THE JOURNAL OF EXPERIMENTAL MEDICINE

THE JOURNAL

OF

EXPERIMENTAL MEDICINE

EDITED BY

SIMON FLEXNER, M.D. PEYTON ROUS, M.D.

VOLUME THIRTY-SIXTH

WITH FIFTY-NINE PLATES AND NINETY-ONE FIGURES IN THE TEXT

NEW YORK
THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH
1922

COPYRIGHT, 1922, BY THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH

Water
Med.
1-20-1923

WAVERLY PRESS
THE WILLIAMS & WILKINS COMPANY
BALTIMORE, U. S. A.

JUL 7 1922 Medical Lib.

THE JOURNAL

OF

EXPERIMENTAL MEDICINE

EDITED BY

SIMON FLEXNER, M.D. PEYTON ROUS, M.D.

VOLUME XXXVI, No. 1 JULY 1, 1922

PUBLISHED MONTHLY

AT MOUNT ROYAL AND GUILFORD AVES., BALTIMORE, MD.

BY THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH

COPYRIGHT, 1922, BY THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH

Entered as second-class matter June 3, 1916, at the Post Office at Baltimore, Md., under the act of March 3, 1879.
Acceptance for mailing at special rate of postage provided for in Section 1103, act of October 3, 1917.
Authorized June 29, 1918.

Made in the United States of America

PUBLICATIONS OF THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH

THE JOURNAL OF EXPERIMENTAL MEDICINE

EDITED BY

SIMON FLEXNER, M.D. PEYTON ROUS, M.D.

The Journal of Experimental Medicine is designed to cover the field of experimental medicine. Information regarding contributions and subscriptions is given in full on the back cover.

THE JOURNAL OF BIOLOGICAL CHEMISTRY

EDITED BY

STANLEY R. BENEDICT, New York, N. Y. LAFAYETTE B. MENDEL, New Haven, Conn.

H. D. DAKIN, Scarborough, N. Y. DONALD D. VAN SLYKE, New York, N. Y.

The Journal of Biological Chemistry is designed for the prompt publication of original investigations of a chemical nature in the biological sciences. It is issued monthly. Each volume consists of as many numbers as are required to make a total of between 500 and 600 pages. The subscription price is $3.00 per volume to subscribers in the United States and Canada, payable in advance; $3.25 to those in other countries. The number of volumes issued yearly averages four. The price of single copies varies; it is given on the back cover of each number. Remittances should be made by draft or check on New York, or by postal money order, payable to *The Journal of Biological Chemistry*, Mount Royal and Guilford Avenues, Baltimore, Md., or Avenue A and 66th Street, New York, N. Y.

THE JOURNAL OF GENERAL PHYSIOLOGY

EDITED BY

JACQUES LOEB, New York, N. Y. W. J. V. OSTERHOUT, Cambridge, Mass.

The Journal of General Physiology is devoted to the explanation of life phenomena on the basis of the physical and chemical constitution of living matter. It is issued bimonthly, one volume of about 600 pages appearing in a year. The subscription price per year (one volume) is $5.00, payable in advance. Single copies cost $1.00. Remittances should be made by draft or check on New York, or by postal money order, payable to *The Journal of General Physiology*, Mount Royal and Guilford Avenues, Baltimore, Md., or Avenue A and 66th Street, New York, N. Y.

STUDIES FROM THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH

The published results of investigations conducted in the Institute, or under its grants, are republished at irregular intervals in volumes designated *Studies from The Rockefeller Institute for Medical Research*. The subscription price is $2.00 per volume, payable in advance. A special rate of $1.00 per volume is made to those who subscribe to *The Journal of Experimental Medicine*, *The Journal of Biological Chemistry*, or *The Journal of General Physiology*. Remittances should be made by draft or check on New York, or by postal money order, payable to The Rockefeller Institute for Medical Research, Avenue A and 66th Street, New York, N. Y.

SEMIANNUAL LIST

The Institute publishes a *Semiannual List* of all papers by members of the staff and those working under grants from the Institute, stating the title and place of publication of these reports. The *Semiannual List* will be sent regularly upon application.

WAVERLY PRESS
WILLIAMS & WILKINS COMPANY
BALTIMORE, U. S. A.

CONTENTS.

No. 1, JULY 1, 1922.

PAGE

MINOT, GEORGE R. Megacaryocytes in the peripheral circulation. Plate 1 1

FLEXNER, SIMON. Experimental epidemiology. Introductory. 9

LYNCH, CLARA J. An outbreak of mouse typhoid and its attempted control by vaccination 15

AMOSS, HAROLD L. Experimental epidemiology. I. An artificially induced epidemic of mouse typhoid 25

AMOSS, HAROLD L. Experimental epidemiology. II. Effect of the addition of healthy mice to a population suffering from mouse typhoid 45

WEBSTER, LESLIE T. Experiments on normal and immune mice with a bacillus of mouse typhoid 71

WEBSTER, LESLIE T. Identification of a paratyphoid-enteritidis strain associated with epizootics of mouse typhoid 97

AMOSS, HAROLD L., and HASELBAUER, PETER P. Immunological distinctions of two strains of the mouse typhoid group isolated during two spontaneous outbreaks among the same stock 107

DU NOÜY, P. LECOMTE. Surface tension of serum. III. Recovery after lowering by surface-active substances. Plate 2 .. 115

WRIGHT, J. HOMER, and CRAIGHEAD, EUGENE M. Infectious motor paralysis in young rabbits. Plates 3 and 4 135

MAURER, SIEGFRIED, and LEWIS, DEAN. The structure and differentiation of the specific cellular elements of the pars intermedia of the hypophysis of the domestic pig. Plate 5. 141

No. 2, AUGUST 1, 1922.

VALENTINE, EUGENIA, and KRUMWIEDE, CHARLES. The loss of hemolytic capacity by a fraction of a culture of a hemolytic streptococcus without change in agglutination characteristics 157

415648

FELTON, LLOYD D., and DOUGHERTY, KATHARINE M. The organotropic, bacteriotropic, and leucocytotropic actions of certain organic chemicals..... 163
SMITH, THEOBALD, and LITTLE, RALPH B. The significance of colostrum to the new-born calf..... 181
SCOTT, W. J. M. Influence of glands with internal secretion on the respiratory exchange. IV. Effect of suprarenal insufficiency in cats..... 199
HARRIS, WILLIAM H., and FRIEDRICHS, ANDREW V The experimental production of periarteritis nodosa in the rabbit with a consideration of the specific causal excitant. Plates 6 to 14. 219
DUVAL, CHARLES W., and D'AUNOY, RIGNEY. Studies upon experimental measles. II. The enanthematous, exanthematous, pyrexial, and leucocytic syndrome produced in the rabbit by intravenous inoculation of blood from cases of human measles..... 231
DUVAL, CHARLES W., and D'AUNOY, RIGNEY. Studies upon experimental measles. III. The symptom-complex in the guinea pig and rabbit following the intratracheal and intravenous injections of filtered nasopharyngeal secretions from cases of human measles..... 239
LEE, FERDINAND C. Changes in the number of small lymphocytes of the blood following ligation of the thoracic duct.... 247

No. 3, SEPTEMBER 1, 1922.

TENBROECK, CARL, and BAUER, JOHANNES H. The tetanus bacillus as an intestinal saprophyte in man..... 261
JONES, F. S., and LITTLE, RALPH B. An epidemiological study of rhinitis (coryza) in calves with special reference to pneumonia..... 273
EBELING, ALBERT H., and FISCHER, ALBERT. Mixed cultures of pure strains of fibroblasts and epithelial cells. Plates 15 to 18..... 285
ORCUTT, MARION L., and HOWE, PAUL E. The relation between the accumulation of globulins and the appearance of agglutinins in the blood of new-born calves..... 291

De Kruif, Paul H. Rabbit septicemia bacillus, Types D and G, in normal rabbits 309
Jones, F. S. The source of the microorganisms in the lungs of normal animals 317
Swift, Walker E., Haggart, Gilbert E., and Drinker, Cecil K. A new method for measuring the pressure in the pulmonary artery. Plate 19 329
Pappenheimer, Alwin M. Experimental rickets in rats. VI. The anatomical changes which accompany healing of experimental rat rickets, under the influence of cod liver oil or its active derivatives. Plates 20 to 28 335
Noguchi, Hideyo. Etiology of yellow fever. XIV. Duration of the protective effect of anti-*icteroides* immune serum after subcutaneous inoculation into animals 357

No. 4, October 1, 1922.

Carrel, Alexis, and Ebeling, Albert H. Pure cultures of large mononuclear leucocytes. Plates 29 to 37 365
Fischer, Albert. A pure strain of cartilage cells *in vitro*. Plates 38 to 40 379
Carrel, Alexis. Growth-promoting function of leucocytes. 385
Fischer, Albert. Cultures of organized tissues. Plates 41 and 42 393
Carrel, Alexis, and Ebeling, Albert H. Action of shaken serum on homologous fibroblasts 399
Boots, Ralph H., and Cullen, Glenn E. The hydrogen ion concentration of joint exudates in rheumatic fever and other forms of arthritis 405
Storm van Leeuwen, W., Bien, Z., and Varekamp, H. On alimentary leucocytosis in its relation to the "*crise hémoclasique*" of Widal 415
Hess, Alfred F., Unger, Lester J., and Pappenheimer, Alwin M. Experimental rickets in rats. VII. The prevention of rickets by sunlight, by the rays of the mercury vapor lamp, and by the carbon arc lamp. Plate 43 427

HESS, ALFRED F., UNGER, LESTER J., and STEINER, JOSEPH M. Experimental rickets in rats. VIII. The effect of Roentgen rays. Plates 44 and 45 447
SMITH, THEOBALD, and LITTLE, RALPH B. Cow serum as a substitute for colostrum in new-born calves 453
HUDSON, WILLIAM A. The iodine content of the blood following thyroidectomy 469

No. 5, NOVEMBER 1, 1922.

BROUN, G. O. Blood destruction during exercise. I. Blood changes occurring in the course of a single day of exercise .. 481
OLITSKY, PETER K., and GATES, FREDERICK L. Experimental studies of the nasopharyngeal secretions from influenza patients. IX. The recurrence of 1922. Plates 46 to 49 .. 501
COWDRY, EDMUND V., and OLITSKY, PETER K. Differences between mitochondria and bacteria 521
FISCHER, ALBERT. Action of antigen on fibroblasts *in vitro*. II. 535
DU NOÜY, P. LECOMTE. Surface tension of serum. IV. Action of temperature. Plates 50 to 53 547
LUNDSGAARD, CHRISTEN, and MÖLLER, EGGERT. Investigations on the oxygen content of cutaneous blood (so called capillary blood) 559
BLISS, WALTER PARKS. Studies on the biology of streptococcus. II. Antigenic relationships between strains of *Streptococcus hæmolyticus* isolated from scarlet fever 575

No. 6, DECEMBER 1, 1922.

FOOT, NATHAN CHANDLER. Studies on endothelial reactions. VI. The endothelial response in experimental tuberculous meningoencephalitis. Plates 54 to 56 607
HOGUE, MARY JANE. *Spirochæta eurygyrata*. A note on its life history and cultivation. Plate 57 617
LONGCOPE, WARFIELD T. Insusceptibility to sensitization and anaphylactic shock 627

CARREL, ALEXIS, and EBELING, ALBERT H. Leucocytic secretions 645
LEAKE, CHAUNCEY D., and BROWN, THOMAS K. The loss of circulating erythrocytes in certain types of experimental pneumonia 661
COWDRY, EDMUND V. The supravital staining of vaccine bodies. Plates 58 and 59 667
OLITSKY, PETER K., and GATES, FREDERICK L. Experimental studies of the nasopharyngeal secretions from influenza patients. X. The immunizing effects in rabbits of subcutaneous injections of killed cultures of *Bacterium pneumosintes* 685
HAGAN, WILLIAM A. Studies on the disease of guinea pigs due to *Bacillus abortus* 697
HAGAN, WILLIAM A. The value of heat-killed cultures for the prevention of the *Bacillus abortus* inoculation disease of guinea pigs 711
HAGAN, WILLIAM A. The susceptibility of mice and rats to infection with *Bacillus abortus* 727
SWIFT, HOMER F. The action of sodium salicylate upon the formation of immune bodies 735
INDEX TO VOLUME XXXVI 761

MEGACARYOCYTES IN THE PERIPHERAL CIRCULATION.*

By GEORGE R. MINOT, M.D.

(From the Collis P. Huntington Memorial Hospital of Harvard University, and the Medical Services of the Massachusetts General Hospital, Boston.)

PLATE 1.

(Received for publication, December 30, 1921.)

Immature forms of red cells and white cells which originate in the bone marrow are commonly observed in the peripheral blood in many pathological conditions. The presence of large numbers of such cells in the blood is a feature of myelogenous leucemia. It might be expected that similarly either true immature forms, or what correspond to immature forms, of the third formed element of the blood, which also originates from the bone marrow, namely the blood platelets, would be present under those conditions in which we find immature red and white cells.

The megacaryocyte (megalocaryocyte) of the bone marrow has been conclusively shown by Wright (1) to be a parent cell of the platelets. The appearance of megacaryocytes in the peripheral blood does not seem to have been noted in the English literature. Isolated observations that they do occur are reported in foreign literature by Oelhafen (2), Di Guglielmo (3), Cesaris-Demel (4), Kaznelson (5), and Nägeli (6). More recently an article has been published by Pianese (7) and another by Di Guglielmo (8) that also refer to the occurrence of megacaryocytes in the circulating blood. These authors found them in cases of myelogenous leucemia, polycythemia vera, and rarely with simple leucocytosis.

It is the object of this report to emphasize that megacaryocytes occur in the peripheral blood and that they occasionally appear in

* This paper is No. 27 of a series of studies on the physiology and pathology of the blood, from the Harvard Medical School and allied hospitals, a part of the expense of which has been defrayed from a grant from the Proctor Fund of the Harvard Medical School for the Study of Chronic Diseases.

large numbers. I have frequently found these cells in the blood of myelogeneous leucemia patients, and have observed them in two cases of polycythemia vera, one of lobar pneumonia, one of Hodgkin's disease, and one of sepsis.

Owing to the normally large size of the megacaryocytes, one can conceive that if they left the bone marrow they would be unable to pass through the finer capillaries of the lungs. There is ample proof, originally given by Aschoff (9) and since by Bunting (10), MacCallum (11), and Wright (1) among others, that megacaryocytes may become packed in the lung capillaries in various conditions calling forth abnormal or immature bone marrow elements.

The presence in the peripheral blood of immature megacaryocyte elements, namely undifferentiated bits of their cytoplasm, together with large numbers of platelets and the subsequent demonstration of megacaryocytes in the lung capillaries, has been noted in Hodgkin's disease by Bunting (10). Cytoplasmic bits of these bone marrow giant cells, which are misnamed giant platelets by Fonio (12) occasionally have been observed in the circulating blood in various other diseases. The presence of these cytoplasmic bits was associated with marked increase of the blood platelets. Variation in the histology of the platelets has received very little attention as compared with that given to the red cells and white cells. Rather marked pathological variation in the size, contour, and character of the granules of the platelets occurs, as shown in studying them in fresh vitally stained as well as fixed preparations. Some such changes in the platelets may be interpreted as indicating immaturity. I have observed frequently abnormal forms and found bits of megacaryocyte cytoplasm in the blood under a wide variety of conditions, including Hodgkin's disease, but notably in myelogenous leucemia. These abnormal derivatives of the megacaryocytes are seen particularly when there is a considerable increase of platelets. The more marked deviations from normal occur when there is other evidence of intense activity of a pathological marrow.

The character of the megacaryocytes seen in the peripheral blood is shown in the accompanying plate.[1] These giant cells have

[1] It is a pleasure to acknowledge that Dr. J. H. Wright has examined some of the preparations from which these cells were drawn and that he considers them to be identical with megacaryocytes seen in the marrow.

distinctive characters more readily observed than described. In the bone marrow the megacaryocyte often characteristically develops a large amount of cytoplasm with ameboid activity so that it projects pseudopods into the blood channels. The formation of platelets results from the segmentation of the pseudopods. Pseudopods not connected with a nucleus are seen not infrequently and, as indicated above, may enter the blood stream. The cytoplasm when stained with Wright's stain contains numerous characteristic red to purple granules, and its edges often appear to be hyaline. Vacuoles occur in the cytoplasm. In the marrow the amount of cytoplasm associated with the megacaryocyte nucleus varies in consequence of the formation of platelets, and nuclei showing the usual signs of degeneration with little or no cytoplasm are frequent. In the peripheral blood the nuclei appear usually to be stripped of their cytoplasm, but as shown in the plate some retain a small amount, which is in the process of differentiation into platelets. In some instances formed platelets may become adherent to the nuclei when the preparation is made. In the bone marrow the nucleus of these cells often appears as a complicated pleomorphic structure that typically is lobate and basket-shaped. Owing to its character the nucleus may appear multiple; if it is actually multiple, this indicates degeneration. The nucleus in the marrow is frequently more vescular than when seen in the blood stream. However, these nuclei seen in the peripheral blood are entirely similar to many of those seen in the marrow. They are of varying shapes and sizes, stain deeply, and present the characteristic spotty internal structure shown in the drawings. At times only a fragment of a nucleus occurs. Many of the nuclei seen in the peripheral blood are smaller than the majority of those seen in the marrow. At autopsy of three cases of myelogenous leucemia there were demonstrated in the lungs many much larger megacaryocytes than were found in the peripheral blood during the last days of life. The lungs of these individuals presented a remarkable appearance. The alveolar walls were thickened and many of the capillaries were plugged with enormous numbers of megacaryocytes. It would seem as if this capillary embolism might have embarrassed the pulmonary circulation and contributed to the dyspnea that these patients had during the last few days of

their life. However, they all had extreme anemia, which could account for their dyspnea. With an increased formation of megacaryocytes diminutive and atypical forms of megacaryocytes are frequently seen in the marrow. Increased numbers and diminutive and atypical forms are characteristic of myelogenous leucemia. This is in harmony with the conception that cells of tumors and tumor-like processes deviate from type and show atypicality. Thus it is not surprising that small megacaryocytes that can pass through the lungs occur in the blood of these cases. Many of the megacaryocyte nuclei observed in the peripheral blood are small, due to fragmentation in consequence of degeneration.

It is not at all uncommon to find an occasional small megacaryocyte (less than 1 per cent of the white count) in the peripheral blood in chronic myelogenous leucemia. During the continued study of some forty-five cases of this disease I have observed at one time or another megacaryocytes in thirty-five cases. In some instances these cells were observed before any form of radiation was given the patient, in others after such therapy. Certain cases showed these cells more persistently than others. When an occasional megacaryocyte was observed the patient was not so well as when they were absent and the white count was usually over 50,000 per c. mm. Even so, many patients showing an occasional one of these giant cells were not particularly sick. In six cases the megacaryocytes occurred in relatively large numbers. Two individuals, one who had had the disease for 4 years and the other for 6 years, showed from 1 to 8 per cent of these cells in white counts of from 50,000 to 150,000 per c.mm. during their last 3 months of life. Three others showed 1 to 4 per cent of these cells in white counts of from 75,000 to 400,000 per c.mm. All five of these cases were sicker than many of those who had an occasional megacaryocyte in their blood. They showed other evidence of unusual activity of the disease at the time the megacaryocytes were present as demonstrated by the presence of fever, a basal metabolism in the vicinity of +60, and numerous myeloblasts and other immature bone marrow cells in the blood stream. The sixth case that showed many of these giant cells was a most unusual one. The patient, who did not appear particularly sick, was a woman of 45 years, with an enormous spleen

and the symptoms of leucemia; before radium treatment she showed a hemoglobin of 75 per cent and a white count of 40,000 per c.mm. Many of these elements in carefully made preparations were broken, indicating their immaturity, and they could not be identified. The striking feature of the differential count of the intact cells was that 18 per cent were megacaryocytes and but 16 per cent myelocytes of various types. Myeloblasts were rare. The remaining cells were largely polynuclear neutrophils. Nucleated red cells were observed occasionally. The platelets were greatly increased. The patient, though continuing to have a large spleen, has been symptomatically very definitely benefited in the past 2 years by radium therapy. During this time the blood has shown no higher white count than 40,000 per c.mm., and seldom more than an occasional megacaryocyte, usually none. The immature white cells have fluctuated considerably in number, as have the platelets.

In two cases of typical polycythemia vera, one of Hodgkin's disease, one fatal case of lobar pneumonia, and one of sepsis, I have found an occasional, usually small, megacaryocyte. The blood in all of these cases showed increases of platelets with bits of megacaryocyte cytoplasm. The two cases of polycythemia were not particularly sick. Both showed various early forms of nucleated red cells and white counts of 25,000 to 40,000 cells per c.mm.; although no undoubted myeloblasts were present, 4 to 8 per cent of myelocytes occurred. The patients with pneumonia and sepsis both had a white count of about 90,000, consisting chiefly of polynuclears, but with a few myelocytes and normoblasts. The case of Hodgkin's disease, that died shortly after megacaryocytes were found in the blood, had a white count of 40,000. The polynuclears were 80 per cent, the large mononuclears 19 per cent, and the eosinophils 1 per cent, and a few normoblasts were seen.

Increase of platelets was the rule when megacaryocytes were found in the blood, but in some instances of myelogenous leucemia megacaryocytes were present when the platelets occurred in normal numbers and rarely when they were decreased. In all the instances in which more than an occasional megacaryocyte was found, there was a marked and usually an extreme increase of platelets, reaching to

over 3 millions in one case. In such cases bits of megacaryocyte cytoplasm were found frequently. When the giant cells were found there was usually other evidence of a serious alteration in the rate of emergence of the cells from the marrow as shown by the presence of many immature cells of both the white and red series, though the white count was not necessarily very high or the anemia marked. In myelogenous leucemia with the white count below 30,000, myelocytes less than 12 per cent, and no myeloblasts, the appearance of megacaryocytes and increases of platelets may be the first sign of increased activity of the disease, as shown in two cases. Usually myeloblasts and primordial nucleated red cells were found, in the cases of myelogenous leucemia when megacaryocytes were present. Of course, as is well known, myeloblasts and primordial nucleated red cells occur without megacaryocytes. None of these three elements normally occurs in the blood stream. Their presence in the blood stream is sufficient to indicate a serious alteration of the regulatory mechanism for the emergence of cells from the bone marrow into the circulating blood. The marrow in such instances is subject to great strain. The pathological process which underlies this altered regulatory mechanism is varied, since these immature cells occur in the blood not only when there are structural changes in the bone marrow, as in myelogenous leucemia, but also with other changes, probably largely functional, as in pneumonia and sepsis.

Apparently when megacaryocytes appear in considerable numbers in the circulating blood, the pathological process in the marrow particularly involves this element. In general the presence of these cells in the blood stream is not to be looked upon as indicative of a desirable type of marrow regeneration but more as indicative of a marrow disintegration, recovery from which may, however, occur.

CONCLUSIONS.

A megacaryocyte is seen commonly as an occasional cell in the peripheral blood of patients with myelogenous leucemia. Less commonly they appear in relatively large numbers.

These giant cells also may occur in the blood under other conditions.

Their presence is indicative of a bone marrow under intense strain.

BIBLIOGRAPHY.

1. Wright, J. H., *J. Morphol.*, 1910–11, xxi, 263.
2. Oelhafen, H., *Folia hæmatol.*, 1914, xviii, 171.
3. Di Guglielmo, G., *Folia med.*, 1917, iii, 386.
4. Cesaris-Demel, cited by Di Guglielmo (3).
5. Kaznelson, P., *Deutsch. Arch. klin. Med.*, 1917, cxxii, 72.
6. Nägeli, *Centr. allg. Path. u. path. Anat.*, 1914, xxv, 433.
7. Pianese, G., *Hæmatol.*, 1920, i, 61.
8. Di Guglielmo, G., *Folia med.*, 1920, vi, 1, 36, 55, 81, 101.
9. Aschoff, L., *Virchows Arch. path. Anat.*, 1893, cxxxiv, 11.
10. Bunting, C. H., *Bull. Johns Hopkins Hosp.*, 1911, xxii, 114.
11. MacCallum, W. G., A text-book of pathology, Philadelphia and London, 2nd edition, 1920.
12. Fonio, A., *Cor.-Bl. schweiz. Aerzte*, 1918, xlviii, 1300.

EXPLANATION OF PLATE 1.

This plate illustrates selected megacaryocytes found in ordinary cover-glass blood smear preparations of the peripheral blood from six different cases. The preparations were stained with Wright's modification of Romanowsky's stain. The drawings were made by the aid of a camera lucida. × 1,300.

The seven megacaryocytes labelled 1 are from a case of myelogenous leucemia. Some are naked nuclei. The larger uppermost nucleus shows some adherent cytoplasm of a megacaryocyte.

The groups of cells labelled 2, 5, and 7 are also from cases of myelogenous leucemia.

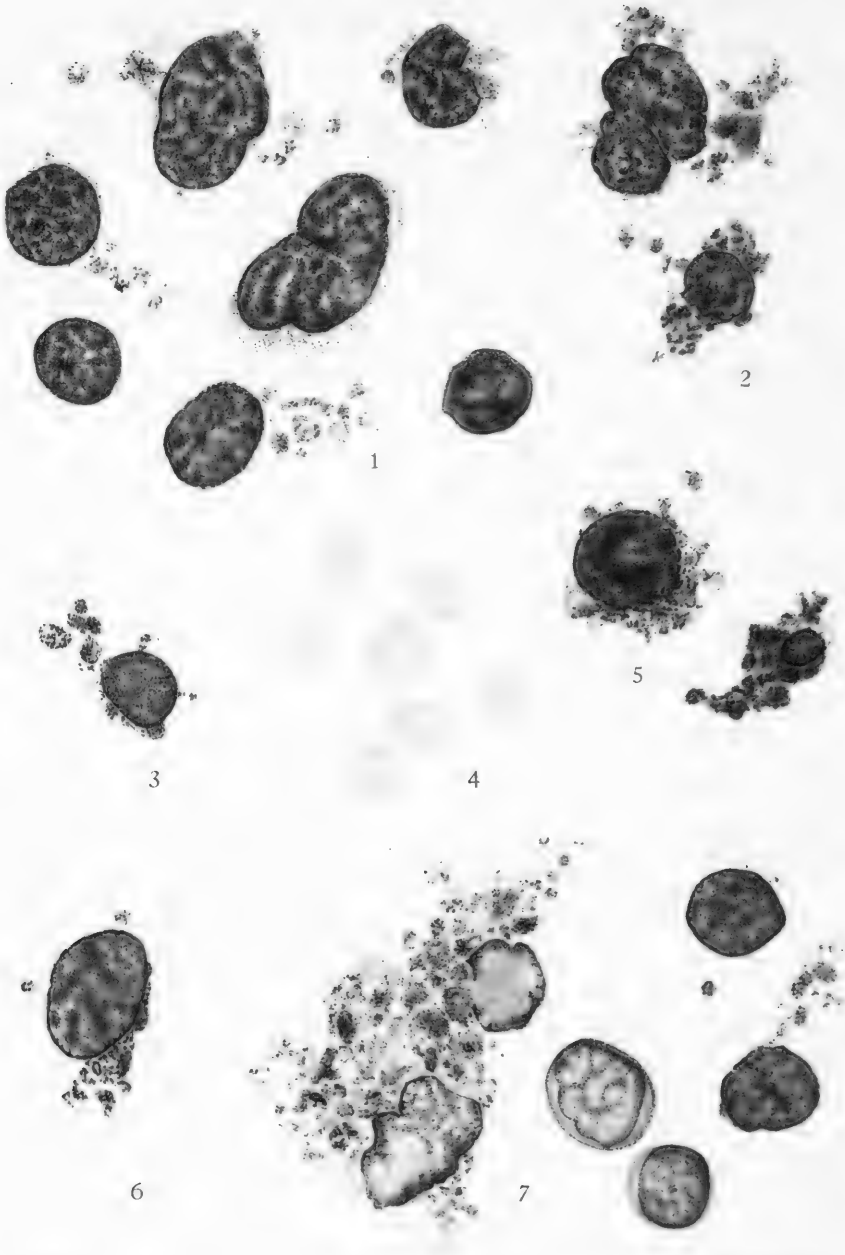
In Group 2 megacaryocyte cytoplasm with its differentiation into platelets is distinct.

In Group 5 is shown a fragment of megacaryocyte nucleus with cytoplasm; differentiation into platelets is taking place.

In Group 7 two myeloblasts (the two central cells) are shown for contrast. On the left the large mass of platelets and megacaryocyte cytoplasm, associated with the somewhat degenerated megacaryocyte nuclei, may have come into approximation with the nuclei in the process of making the preparation.

The small megacaryocyte in Group 3 occurred in a case of lobar pneumonia, while that shown in Group 6 is from the blood of a case of polycythemia vera.

The red cells (Group 4) are inserted to illustrate the comparative size of the megacaryocytes.



E. Piotti,—20

A. HUEN & CO. BALTIMORE

(Minot: Megacaryocytes in peripheral circulation.)

EXPERIMENTAL EPIDEMIOLOGY.

Introductory.

By SIMON FLEXNER, M.D.

(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

(Received for publication, May 15, 1922.)

Each generation receives its particular impression of epidemic diseases; during the past 20 years the impressive epidemics of the western world have been those of meningitis or cerebrospinal fever, poliomyelitis, influenza, and latterly lethargic or epidemic encephalitis. These several epidemic diseases have numbered their cases by the hundreds, thousands, and even tens of thousands in given communities or countries, and have ravaged not only the United States, but within the same period have prevailed, sometimes with more, sometimes with less intensity, in other and distant parts of the world.

All these epidemics are indeed old-world diseases, and excepting the last, namely lethargic encephalitis, regarding which knowledge is still scant and uncertain, they can be traced far back in recorded human history. Moreover, the remarkably wide or pandemic outbreaks of which the recent American epidemics form part all originated in the old world and were communicated, usually after a lapse of time, to the new world, where, in some instances, as in the notable one of the 1916 wave of poliomyelitis, they found a soil so fertile and circumstances of spread so favorable as to reach a height of destructiveness previously unknown.

Ever since Hippocrates and especially since Sydenham, the study of epidemics of disease with a view to penetrating their hidden meaning has engaged the attention of occasional men. The degree of interest in what may be called the nature of epidemics has, however, fluctuated greatly and considerable periods have elapsed in which the subject has been given only superficial thought. Then

circumstances have arisen through which both the professional and the public mind has become so engrossed with it that new efforts come to be put forth in order to grasp its significance.

We are moving now in such a period of revived interest. Ever since the rise of modern bacteriology, the hope has been indulged that as knowledge of the microbic incitants of disease grew, the nature of epidemics would become more comprehensible. That this hope has not been entirely disappointed is shown by the influence which precise knowledge of certain disease-producing microorganisms, taken directly into the gastrointestinal tract or communicated by insect vectors, has had on the control of maladies thus induced. The discoveries in these two fields alone and the measures derived from them, as well as the discovery of the part played by those potential distributors of the microbes of disease called "carriers," have led to practical achievements of great magnitude in the domain of hygiene, imparting a sense of human power and control over impending epidemics of disease, in profound contrast with the helplessness of any previous period in history.

And yet in certain quarters, and especially in England, what may be defined as a reaction against the teachings of modern, that is bacteriological, epidemiology seems to have set in. That this return to older, and as it must seem, more mystical doctrines is widespread cannot be affirmed, but it has even happened that in the effort to elucidate the epidemic prevalences of the past two decades already enumerated and eventuating in the colossal outbreak of influenza, the notions of epidemic constitutions as defined by Hippocrates and especially by Sydenham have been more or less sported with, if not actually invoked.[1]

[1] Payne, J. F., Thomas Sydenham (Masters of medicine series), London, 1900. Greenwood, M., Sydenham as an epidemiologist, *Proc. Roy. Soc. Med.*, 1918–19, xii, Epidemiol. and state med. sect., 55. Goodall, E. W., Discussion on Sydenham, *Proc. Roy. Soc. Med.*, 1918-19, xii, Epidemiol. and state med. sect., 66. Singer, C., Discussion on Sydenham, *Proc. Roy. Soc. Med.*, 1918–19, xii, Epidemiol. and state med. sect., 71. Greenwood, M., The factors that determine the rise, spread and degree of severity of epidemic diseases, *XVII Internat. Cong. Med.*, 1913, sect. 18, 49. Report on the pandemic of influenza, 1918–19, *Ministry of Health, Rep. Pub. Health and Med. Subj., No. 4*, 1920.

To the extent to which this reaction is an endeavor to define epidemiology in terms wider than those of the microbic incitants of disease alone it may be regarded as wholesome and timely. The doctrine of "carriers" of potentially infecting microorganisms necessitates a conception of epidemic disease wider than is embraced in the exclusive view of their varying pathogenic activities. That the mere occurrence of potent microorganisms does not suffice to produce an outbreak of epidemic disease is a commonplace of bacteriological knowledge. The conditions are not as simple as that, and in their greater complexity they include not only the various qualities of the microbe but also of the host and, as well, their many reactions, one upon the other.

But it may be questioned whether progress is to be gained by a return to the indefinite concepts of epidemic constitutions or of the interdependence of diseases as various as epidemic meningitis, poliomyelitis, influenza, etc., united by an inevitable nexus of events the nature of which cannot even be surmised.[2]

Against these vague considerations modern bacteriology opposes the belief in a specific etiology, in which a particular microorganism is sought as the incitant of the infectious disease under study. The triumphs already secured in this field need not be recited here. But it is obvious that the gains made are not sufficient to account for all the phenomena of epidemics. The search, therefore, must, if possible, be both widened and deepened. Hence, the immediate question which presents itself is the manner in which this can be done.

[2] Hamer, W. H., The Milroy lectures on epidemic disease in England—the evidence of variability and of persistency of type, *Lancet*, 1906, ii, 569, 655, 733; Discussion on influenza, *Proc. Roy. Soc. Med.*, 1918–19, xii, Roy. Soc. Med. sect., 24; Discussion on Sydenham as epidemiologist, *Proc. Roy. Soc. Med.*, 1918–19, xii, Epidemiol. and state med. sect., 72. Newsholme, A., and Stevenson, T. H. C., et al., Discussion on influenza, *Proc. Roy. Soc. Med.*, 1918–19, xii, Roy. Soc. Med. sect., 1. Crookshank, F. G., First principles: and epidemiology, *Proc. Roy. Soc. Med.*, 1920, xiii, Epidemiol. and state med. sect., 159; A note on the history of epidemic encephalomyelitis, *Boston Med. and Surg. J.*, 1920, clxxxii, 34; Public health considerations relating to influenza, pneumonia, and allied epidemics. The epidemiological point of view, *Boston Med. and Surg. J.*, 1921, clxxxiv, 548. Report on the pandemic of influenza, 1918–19, *Ministry of Health, Rep. Pub. Health and Med. Subj., No. 4*, 1920.

Epidemic outbursts of disease occur among animals and pursue a course similar to that which has been observed to occur in man. The direct study of epidemics among animals under conditions of control not attainable in man should therefore commend itself to the epidemiologist. By this means it may be possible to secure those precise data of both microorganism and host on which eventually a real science of epidemiology may come to be built.

Thus an investigation was undertaken several years ago relating to certain aspects of experimental epidemiology of which the papers to follow are the first fruits. The papers relate to epidemics in mice of gastrointestinal origin, to which the name of mouse typhoid is applied. While mouse typhoid presents clinical and pathological characteristics of a single disease-complex, its microbic incitant is not a consistent species. Indeed, just as there are distinct but related bacilli inducing dysentery in man, there are distinct but related bacilli capable of provoking "typhoid" in mice. This relative multiplicity of microbic factors may be viewed as an aid rather than as a hindrance in investigating the events taking place in course of epidemics, the knowledge of which may come to have applicability to man as well as to the lower animals. The several bacilli alluded to differ little in cultural, but profoundly in immunologic properties. Some of the species exist either as saprophytes or at least as harmless "carried" bacteria in mice regarded as normal and yet become substituted for the original inciting microbes during epidemics—a fact of high importance in respect to the vagaries noted in the bacteriologic investigations of certain epidemics in man.

Not only do specific differences exist among so called mouse typhoid bacilli, but all the pathogenic varieties appear extremely labile. Strains of the bacilli artificially enhanced as they pass from mice to mice quickly fall to an average of infectivity and are, as it seems, at low pathogenic ebb at the time of the death of the infected animals. But this lability of the bacilli is determined, in part, by the hosts; that is, the mice through which they pass. In this respect mice may be viewed as consisting of different biological classes according as they respond to ingestion of the bacilli with infection and death, with mere carriage of the bacilli, or with non-reactibility. The distinctions of classes are not, however, absolute, but are determined, partly at

least, by the quantity or dosage of the bacilli. It is this latter factor which plays so conspicuous a rôle in the phenomenon of recurrent epidemic waves superinduced by the introduction of new mice in the replacement experiments described.[3] While it is the "carrier" among the old mice which provides the "seed" for the next following epidemic outbursts, it is the highly susceptible individuals among the new which furnish the living "culture" medium enabling rapid increase and wide dissemination of the bacilli to be effected, just as it is the succumbing and non-reactible mice which check the growth and multiplication that tend to arrest the epidemic spread.

These factors leave out of account the effect, if any, of active immunization acquired through previous exposure, a condition shown experimentally to be realizable, but the extent and sufficiency of which in initiating the events and finally in bringing about the state of equilibrium between parasite and host prevailing at the end of the epidemic wave are still undetermined.[4]

The papers in this series relate to a gastrointestinal infection in mice. In man the prevalent type of epidemic disease has altered notably within a century; there has been far less of the diseases in which the mode of infection is enteric and more of the diseases in which the portal of infection is by way of the respiratory tract, which fact leads to the consideration that the control which modern hygienic practises exercise over epidemics depends on the imposition of general or communal, as opposed to individualistic measures of prevention. Smallpox is controlled by essentially universal vaccination; typhoid and allied fevers by water purification and similar means; malaria, typhus, and yellow fever by war on their insect propagators. While conversely the epidemic diseases which still prevail almost unchecked among the western peoples are just those for which no communal means, practically applicable, exist of preventing communication of the potentially morbid materials, such as those of the secretions of the respiratory tract, from individual to individual. And coinci-

[3] Amoss, H. L., Experimental epidemiology. II. Effect of the addition of healthy mice to a population suffering from mouse typhoid, *J. Exp. Med.*, 1922, xxxvi, 45.

[4] Webster, L. T., Experiments on normal and immune mice with a bacillus of mouse typhoid, *J. Exp. Med.*, 1922, xxxvi, 71.

dentally with this circumstance, other significant conditions have arisen to modify previous history, namely the rise of multitudinous cities, industrialism, rapid transport, etc., the effects of which are to increase the number and intensity of personal contact associations, and thus to combine, confuse, and distribute quickly and widely the respiratory secretions of unnumbered persons.

It is our intention to extend the experimental investigation of epidemics among laboratory animals to the respiratory-borne infections. Opportunities for such studies undoubtedly exist. The obvious differences and inconsistencies between the animals thus affected and man, primarily in respect to the distinction in habits, may not prove insuperable obstacles in the way of obtaining illuminating information from the one applicable to the other. In any case it will be found desirable to check the experimental studies on epidemics of enteric origin with those of respiratory origin and to determine in how far they pursue similar and to what extent they follow different modes of evolution and devolution.

AN OUTBREAK OF MOUSE TYPHOID AND ITS ATTEMPTED CONTROL BY VACCINATION.

By CLARA J. LYNCH, PH.D.

(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

(Received for publication, January 28, 1922.)

In this paper will be described briefly an epidemic of so called mouse typhoid which prevailed with fluctuations for approximately $2\frac{1}{2}$ years in a mouse breeding station maintained at The Rockefeller Institute.

The original stock of about 3,000 mice of all ages was procured by purchase and transported *en masse* to The Rockefeller Institute. The stock was especially valuable because it yielded a relatively high percentage of mice developing so called spontaneous cancers, located in the mammary glands chiefly, but also in other organs. It is known that the original stock, from which that purchased by The Rockefeller Institute was derived, had previously suffered losses from mouse typhoid. Moreover, to the stock as purchased, there were added from time to time from the outside a mouse showing a spontaneous tumor or other small accessions of healthy stock.

The transfer of the mouse stock took place in April, 1918. Nothing especially noteworthy in the fatalities arose to arrest attention until about the middle of September of that year, when an unusual number of deaths among the stock occurred.[1] From this time on, until what may be called the termination of the epidemic period, some $2\frac{1}{2}$ years later, the number of deaths may be regarded as having been abnormally high, although in intervals between the wave-crests, the death rate returned to a level as low as that usually regarded as normal.

It was customary to record all deaths occurring in the stock mice and at the beginning of each month to take a census of the population

[1] The summer death rate immediately preceding the September rise was slightly higher than usual. But as during the warm summer period previously a rise had been observed, this increase was not considered significant.

which fluctuated in number roughly between 2,500 and 4,000 individuals. The numbers as given by the census varied according to the matings and to the demands made on the stock for purposes of investigation.

It was the custom also to make a gross postmortem examination of every mouse found dead. When the deaths became so frequent as to excite apprehension of an impending epidemic, bacteriological studies were made. Cultures of the spleen and liver yielded a bacillus which was identified as belonging to the group of mouse typhoid bacilli. During the course of the bacteriological studies about a dozen strains conforming to the cultural characteristics of the group were isolated. Of this number two strains were retained in culture and transplanted regularly and thus kept alive. But it was not until about a year after the epidemic began that the two strains were subjected to immunological study by Dr. Amoss.

The two strains proved to be identical in cultural and immunological reactions, and there was no reason to suppose that the first epidemic was induced by more than this one strain of a bacillus belonging to the enteritidis group. As this strain was utilized by Dr. Amoss in certain of his experiments on the production of artificial epidemics among mice,[2] its more precise biological description will be found in his papers.[3] For sake of convenience the strain has been designated Mouse Typhoid I.

Before proceeding to the more detailed account of the epidemic among the stock of cancer mice, as they were called, a brief statement regarding the conditions under which mice are propagated at The Rockefeller Institute may be of interest.

Two distinct breeding rooms or stations for mice are maintained, with separate caretakers who do not mingle. One station houses the cancer stock and the other the normal stock. The latter has been inbred for the past 2 years, no outside accessions having been made during this period, and is employed in the general investigative work of the Institute. The completeness with which separation between the two stations has been effected is indicated by the fact that in the long period during which deaths—many or few—from mouse

[2] Amoss, H. L., *J. Exp. Med.*, 1922, xxxvi, 25.

[3] Amoss, H. L., and Haselbauer, P. P., *J. Exp. Med.*, 1922, xxxvi, 107.

typhoid were taking place among the cancer stock there were only four deaths among the normal stock apparently from this cause. The population of the normal stock was kept approximately at from 2,500 to 3,000 individuals.

The Epidemic.

The course of the epidemic outbreak can best be followed by observing the graphic curve which is reproduced in two sections which have been subdivided into segments (Text-fig. 1).

As the first segment (No. 1) of the curve indicates, the death rate among the mice rose in the 2nd week of September and actually became about twice the usual weekly rate. From this time on the records were kept more accurately than before and they show a gradual rise in the number of deaths up to the 1st week in November, when the rate remained constant for 10 days, increased during the 3rd week, and began to decline abruptly.

The curve indicating actual number of deaths forms a plateau extending over a period of 2 weeks during which there was a slight depression. The decline was slightly more irregular than the rise.

The course of the epidemic is perhaps better described by the attack rate per 1,000 of population, which rose sharply until November 19 and declined as rapidly as it had risen.

The return to what is regarded as the usual death rate came about January 18, 1919. The outbreak lasted about 140 days; the peak was reached at about the middle of this period, or the 80th day (November 19), so that the curve representing the rate per 1,000 is fairly symmetrical with a slight lag in the decline.

From a consideration of the character of the curve during December, there is a suggestion of the occurrence of another small wave which might have begun somewhere between December 15 and 20 and reached its peak on December 29. The interval between the peaks is about 15 days. Such a supposition is strengthened by the occurrence of a similar wave in March (shown in Segment 2) which endured for 15 days. It is not improbable that the total outbreak is really a series of six overlapping epidemic waves, each lasting about 15 days. Unfortunately no spot map was kept recording the distribution of the deaths in the breeding station. In general the epi-

TEXT-FIG. 1. Segment 1. Deaths from all causes among the cancer breeding stock by 5 day periods from September 5, 1918, to January 18, 1919.

Segment 2. Deaths from all causes by 5 day periods from January 19 to April 30, 1919.

Segment 3. Deaths from all causes by 5 day periods from May 1 to September 30, 1919.

Segment 4. Deaths from all causes by 5 day periods from October 1, 1919, to February 29, 1920.

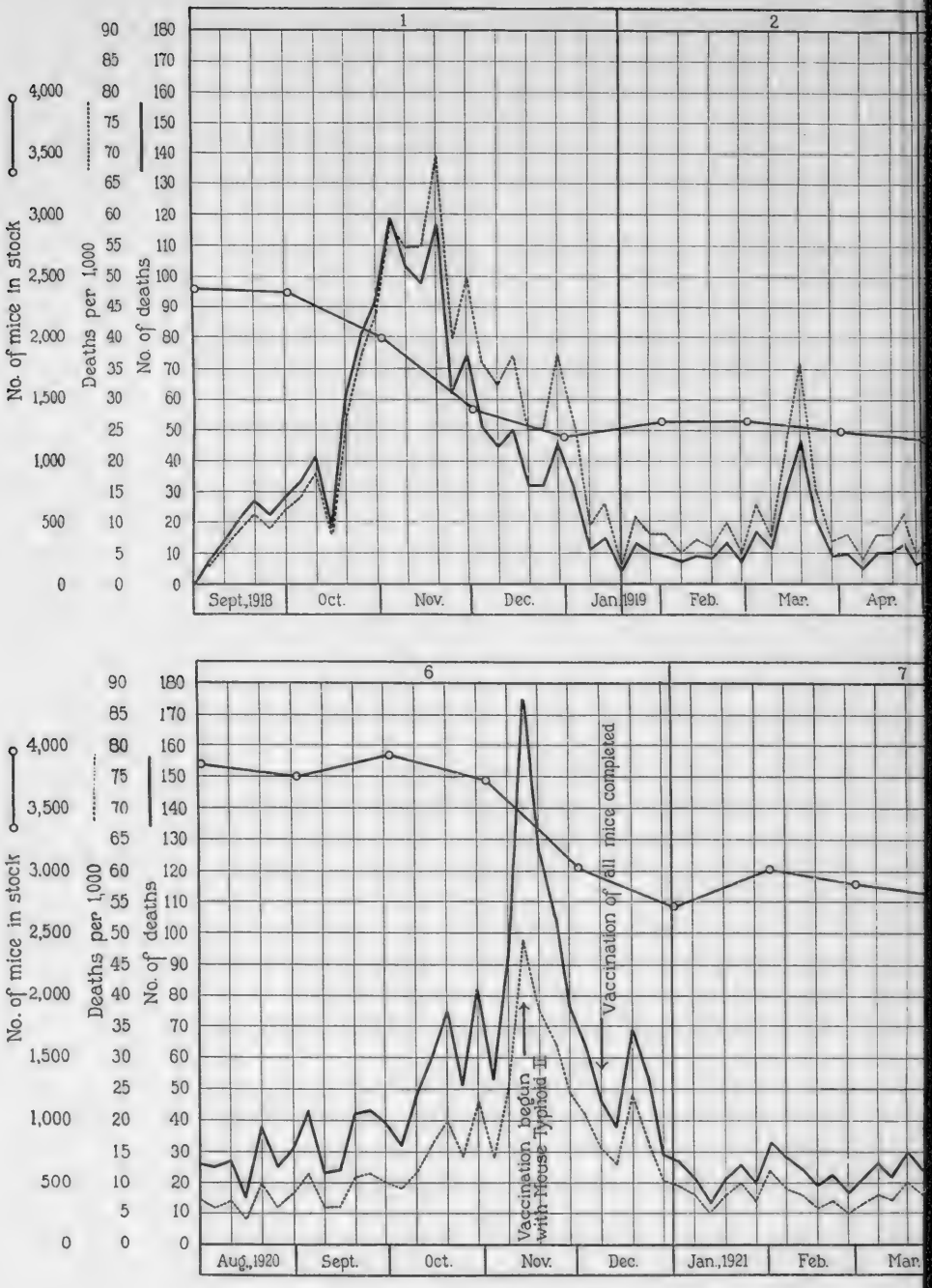
Segment 5. Deaths from all causes by 5 day periods from March 1 to July 31, 1920.

Segment 6. Deaths from all causes by 5 day periods from August 1 to December 31, 1920.

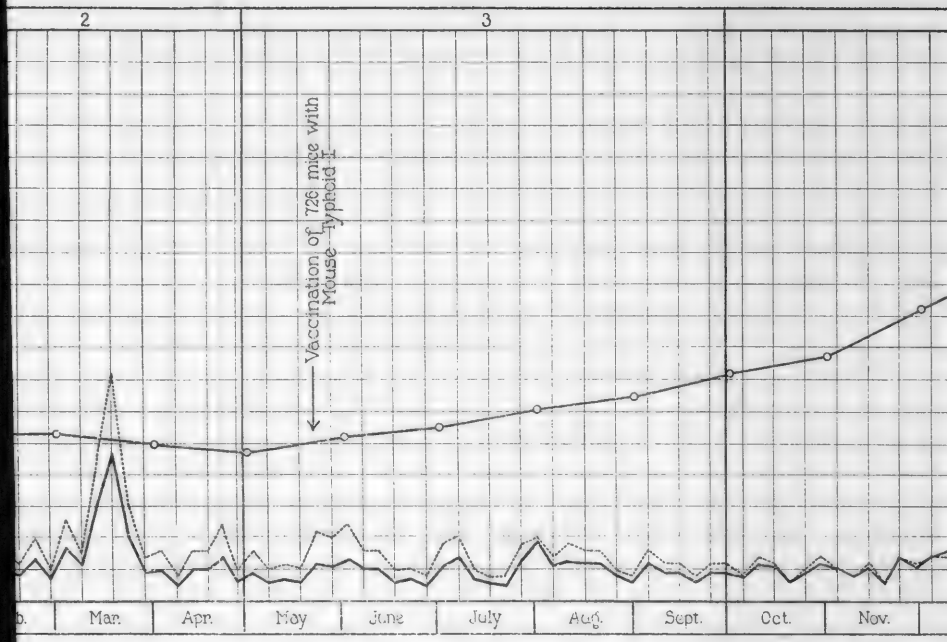
Segment 7. Deaths from all causes by 5 day periods from January 1 to May 31, 1921.

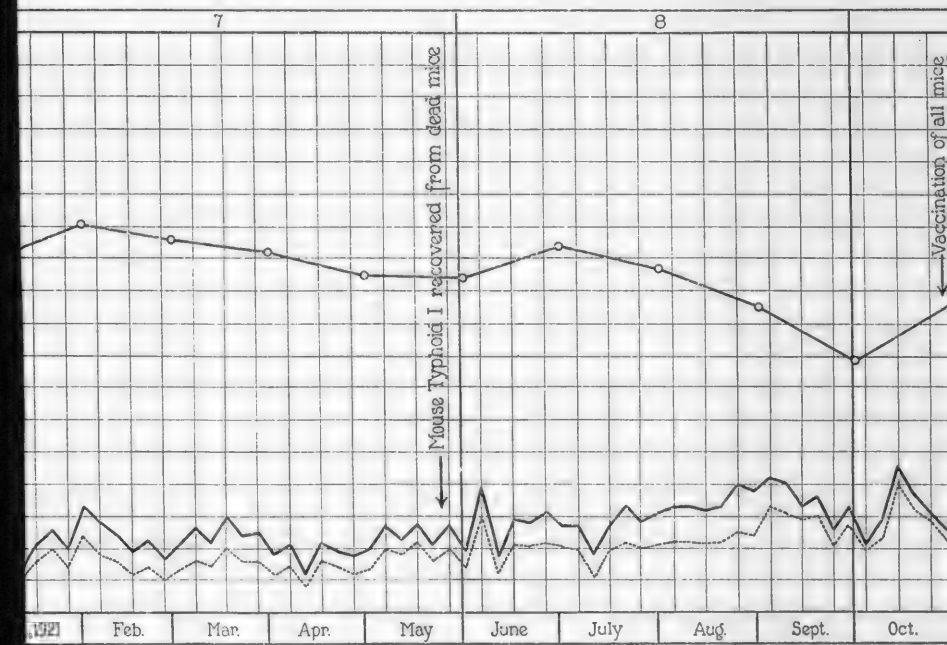
Segment 8. Deaths from all causes by 5 day periods from June 1 to September 30, 1921.

Segment 9. Deaths from all causes by 5 day periods from October 1 to December 31, 1921.

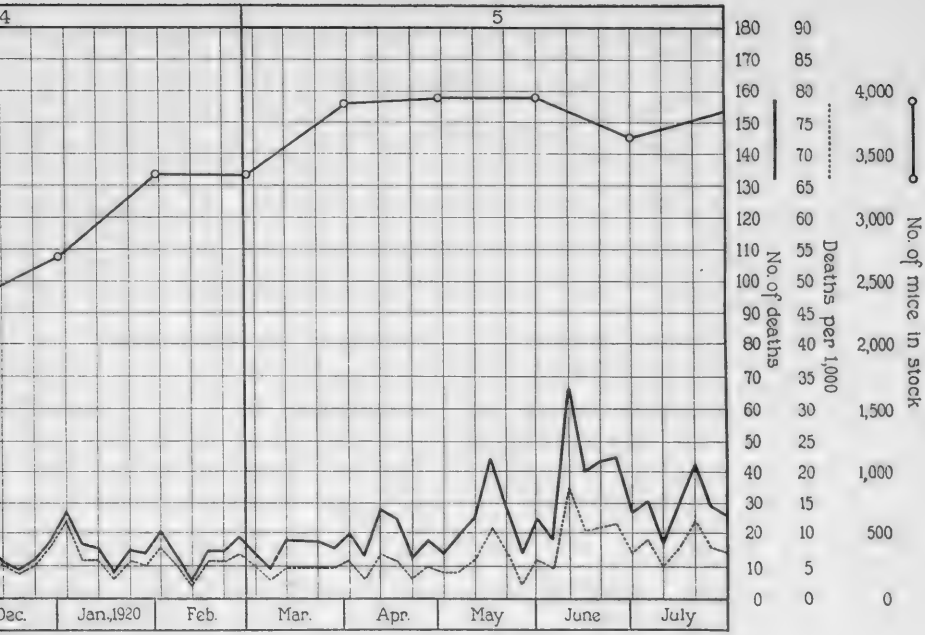


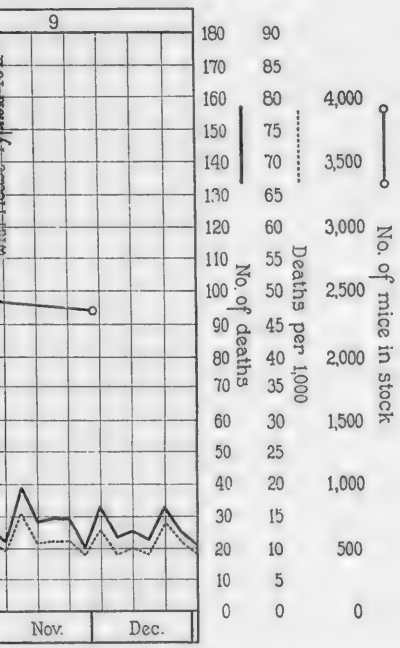
TEXT-FIG. 1. ----------Deaths per 1,000 of mouse population by 5 day perio





opulation by 5 day periods. —— Actual number of deaths in mouse population by 5 day periods





——— Total number of mice in stock according to the monthly census.

demic raged on the shelves where the older mice were kept. For example, among 1,465 older mice the mortality was 82 per cent, whereas among 1,034 new mice, most of which were weaned before the onset of the epidemic, the mortality was 43 per cent.

The severity of this first, or as it may be called November epidemic wave, is shown by the number of deaths presumably in all instances from mouse typhoid. This number as calculated for the period from September 10 to January 28 inclusive is 1,351 mice, or a little more than one-half of the population of the breeding station. During December the deaths occurred mainly in boxes which had already been attacked. Instances of spread to new boxes were frequent.

It is quite certain that this large number of deaths was brought about by the mouse typhoid infection, for while not every dead mouse was studied bacteriologically, yet all were submitted to autopsy and the usually obvious signs of the disease detected.

Along with the epidemic there occurred as a concomitant effect a large reduction in the birth rate so that the number of mice diminished through death was not augmented by births. Hence during the next period of January, February, and 1st week in March, as shown by the second segment (No. 2) the death rate, while still high as compared with the pre-epidemic period, kept quite uniform. However, during the 1st week of March an upward tendency is evident, which at the beginning of the 2nd week is converted into a steady rise culminating in a peak lower than the November peak, enduring a shorter time, and falling more sharply, as the average death rate is again reached in the 1st week of April.

This second, or March epidemic wave is to be considered in the light of two classes, as it were, of the mouse population; namely, the old population which had passed through the November epidemic, and the accessions through new births. The total population had been profoundly reduced by the November epidemic and not yet restored by new births. The March wave was of short duration (15 to 20 days) and was approximately half as severe as that of November. As already stated, the November outbreak may be looked upon as a series of waves following closely one another, whereas the March epidemic represented a single wave.

A period of relative quiescence now set in, as shown by Segment 3. On May 17 the total mouse population was about 1,250 individuals. During the next 4 weeks, half of the mice were given (vaccinated) under the skin of the back a single injection of 0.2 cc. of a suspension (or about 600,000) of killed bacilli of the strain of mouse typhoid isolated during the epidemic. The vaccination produced no immediate effect on the death rate, although it is noteworthy that during a 4 weeks period of mid-May to mid-June, only two of the vaccinated mice died and in only one was the mouse typhoid bacillus found.

The carrying out of the vaccination would seem rather to have coincided with than to have been responsible for the quiescent period which extended from June, 1919, to about January, 1920, the level of which is shown in Segments 3 and 4. During this period the mouse population was rising steadily chiefly through new births. The new individuals were left with the old and no further vaccination was carried out in the breeding station. Although the general death rate had not fallen to that of the pre-epidemic period, yet the new equilibrium which had been established was regarded as satisfactory.

However, beginning in January, 1920, as the population continued to increase, a small rise in death rate also occurred as is shown in Segments 4 and 5. By May, 1920, the total population in the breeding station had reached approximately 4,000. As presaging of the next epidemic, events may be detected in the rise in number of deaths in April, May, and June, as indicated in Segment 5. Although the population had again become large, this mere numerical increase was followed with some anxiety.

The number of deaths per 1,000 continued generally high during July, August, and September. Beginning the 1st week in October, a further rise took place that with minor and perhaps unimportant fluctuations led into the sharp epidemic wave of November, 1920, which exceeded in actual number of deaths that of the first, or 1918 November wave, but the rate per 1,000 was only five-sevenths of that recorded in the 1918 outbreak. The course of this epidemic wave which was practically obliterated by January, 1921, is shown in Segment 6. The wave-like character of the curve is also present. Whereas the epidemic of 1918 seemed to be a summation of six waves, the 1920 outbreak consists of only four, each of which was longer and lower than in the former.

An analysis of this epidemic wave yields the following data. The total mouse population affected by this epidemic wave during its entire course was 4,282, of which 1,463 died. Hence the gross mortality was 34 per cent. Of the 4,282 mice in the total population, 220 were at least 18 months old and had thus passed through the previous severe epidemic wave, while 4,062 were new individuals not so severely exposed. Taking the two classes of the population separately, the old mice (18 months old at least),[4] and the new mice as above described, it may be stated that of 220 of the former, 130, or 60 per cent, succumbed in the November, 1920, epidemic wave, while of 4,062 of the latter, or new stock, only 1,333, or 32.8 per cent, fell victims. The effect of the epidemic on the birth rate was marked. During the extreme height and the following wave in November and December, very few births took place. By January 1, 1921, the total population had fallen to 2,731 and the sharp outbreak may be regarded as having come to an end. For the succeeding 5 months (January to May) the death rate remained at the average low level ordinarily observed in stocks of mice (Segment 7). The usual[1] slight increase in death rate was observed during the summer (Segment 8) and autumn (Segment 9).

There is practically no difference in the seasonal distribution of the two major outbreaks. Each was preceded by a slightly increased death rate during the summer months and began to gain headway in September, reaching the peak on November 19, 1918, and November 13, 1920, respectively. Since the death rate is calculated on a basis of 5 day periods, the difference of 6 days is probably within the limit of error. The entire duration of the 1918 outbreak was about 140 days and of the 1920 epidemic 125 days.

The bacteriology of the November, 1920, outbreak has particular importance. Practically all the dead mice were examined post-mortem, and cultures of the mouse typhoid bacillus were obtained by Mr. Sturm from approximately 75 per cent of the examined animals. Of the many cultures thus obtained, two strains were retained and eventually turned over to Dr. Amoss for use in his experiments.

[4] The average longevity of mice in non-infected stock is about 2 years. Old age must be considered as contributory to a high death rate in such a group.

The immunological study by Dr. Amoss of these cultures showed that they differed from the original cultures isolated in the 1918 epidemic. A full description of this bacillus will be found in another paper of this series.[3]

The fact that the two strains of the paratyphoid-enteritidis group, differing immunologically from each other and both potentially capable of setting up severe epidemics among mice, were responsible for the epidemics separated from each other by 2 years, comes to have a special interest and may possess a particular significance in view of the vaccinations carried out in the period between the two epidemics. For superficially, at least, it appears that the inoculation of the killed cultures of the first bacillus shunted, as it were, that particular organism out of action while leaving the recruited population, both old and new, and the old even more than the new, subject to a second variety of the mouse typhoid bacillus. If this is at all a true statement of what has taken place in the second November epidemic, then the vaccination of part of the surviving population in May and June, 1919, was sufficient to protect the entire population from infection with the first variety of the mouse typhoid bacillus. This last point is obviously one that is open to experimental inquiry as is also the collateral point whether cross-immunity reactions occur between the two varieties of the *Bacillus enteritidis* with which we have been dealing.

No further outbreak of mouse typhoid of an epidemic character has taken place in the cancer breeding station since January, 1921. Just as a large part of the surviving mice was vaccinated after the November, 1918, epidemic wave, so vaccination of all the survivors was carried out at the height of the November, 1920, epidemic wave. The vaccine employed in 1920 was identical with that employed in 1919, and as the two varieties of inciting bacilli differ immunologically, it may be regarded as at least questionable whether the second inoculations have had anything to do with the relative quiescence of the mouse typhoid infection in the breeding station following the last November epidemic.

A slight increase in the death rate occurred during the summer of 1921, and since Mouse Typhoid I had been recovered from some of the mice dying in May the entire population was vaccinated with Mouse Typhoid I and II in October.

Carriers.

No extensive study was made of carriers. In January, 1922, ten mice which had passed through the second epidemic and ten mice born of these were killed and cultured. The cultures were negative for Mouse Typhoid I and Mouse Typhoid II, except for those from one mouse in the former group. In this mouse Mouse Typhoid I was recovered from the cecum only. Cultures from the small intestine were negative for Mouse Typhoid I and Mouse Typhoid II.

EXPERIMENTAL EPIDEMIOLOGY.

I. An Artificially Induced Epidemic of Mouse Typhoid.

By HAROLD L. AMOSS, M.D.

(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

(Received for publication, February 9, 1922.)

The problem set ourselves was the study of an epidemic of mouse typhoid conducted under experimental conditions. The painstaking observations recorded in Dr. Lynch's paper,[1] far exceeding those usually accorded outbreaks of disease among domesticated animals, seemed to put rather than to answer questions of epidemiology. It appeared to us therefore that an epidemic started under fixed conditions, in which one person followed the happenings day by day and recorded the events, would not only tend to eliminate errors traceable to the elements of surprise and lack of preparation, but also by providing a more homogeneous material yield results of greater consistency, while the two major factors of host and parasite would be placed under highly favorable conditions of control.

Method.

Of laboratory mammals, mice are most easily assembled and observed in large numbers. Moreover, they are subject to a bacterial infection, mouse typhoid, of gastrointestinal origin, the pathology of which is quite well known. The lesions found in and characteristic of the disease affect several important viscera and are obvious to the unaided eye. The bacteriology also is sufficiently worked out so as to serve as a guide in what may be regarded as doubtful instances. Moreover, as is well known, the disease mouse typhoid constitutes a common sporadic fatal epidemic affection among mice and even from time to time sweeps through mice colonies in highly destructive waves.

[1] Lynch, C. J., *J. Exp. Med.*, 1922, xxxvi, 15.

In view of the experiences described in Dr. Lynch's paper,[1] our experimental investigations were started with mouse typhoid. While under way, Topley's[2] series of valuable papers on the same general topic began to appear in print. They will be discussed along with the deductions from our own observations in the proper place.

The procedure adopted by us at the outset was one chosen to simulate the conditions of epidemic outbreaks of disease not only among carefully segregated small domestic animals but also those which occur among human beings.

Thus what may be termed a mouse village was set up by placing in rows on metal shelves metal cages 7 by 10 by 5 inches with wire mesh tops. 5 mice were placed in each cage without communication between the cages, so that infection could be transferred only by the hands and implements of the person cleaning the cages and feeding the mice. Great care was taken throughout the experiment to exclude roaches, ants, flies, and other insects and vermin from the room. The temperature of the room was kept constantly at 68°F. whenever the outside temperature was below this. In the summer months the temperature of the room was, of course, higher. The cages were thoroughly cleaned once each week, always in the same order, and the mice were fed in the same order daily as follows: grain in the morning and bread moistened with milk in the afternoon. Precautions were taken to exclude extraneous disease. The mice used came from a carefully controlled healthy stock bred in the Institute and free of communicable disease.

There are certain advantages in keeping the experimental animals in small groups: it simplifies identification and stock taking, and the keeping of records; and allows frequent inspection for dead animals; it reduces the death rate resulting from fighting. The spread of infection is limited to one agent; *viz.*, the cleaning implements and hands of the caretaker.

Introduction of the Virus.—As already stated, when the study was begun Topley's papers had not appeared. His method of placing a known amount of culture on small bits of bread to be consumed by mice from which food had been withheld for 24 hours is efficacious

[2] Topley, W. W. C., *J. Hyg.*, 1920–21, xix, 350.

and more accurate than our first attempts by allowing hungry mice to drink milk from a tube. In order to give the exact dose to each mouse, we later introduced the bacteria suspended in milk directly into the stomach through a small silver tube. In this way the dose can be accurately measured and the use of more than 1 cage for each 5 mice obviated for the preliminary step of infecting the first mice.

Inspection.—The cages were examined three times daily except Sunday, when only one inspection was made. Even under these conditions it sometimes happens, especially on the first round of the day, that only a partially eaten body of a mouse is found. The survivors quickly attack the body of a dead mouse and devour the softer parts. Earlier in the experiments, when we had little experience, the number of deaths which could not be definitely determined as due to *Bacillus typhi murium* infection was proportionately larger than in the later series.

The bodies collected at 9 a.m. rounds were autopsied at 10 a.m. Those collected later were kept in the refrigerator (+4°C.) until the following morning.

Records.—In following the progress of the infection, two charts were kept: (*a*) plot of the total number of deaths for the whole series, according to days, and (*b*) spot map using colored pins showing the location of the mice which died and the number of days elapsed since the beginning of the exposure.

Autopsy Technique.—The notes at autopsy included the weight of the body and of the spleen, and a brief description of the macroscopic appearances of the spleen, intestines, liver, and gall bladder. The bacteriological examinations of the spleen, intestinal contents, and gall bladder were carried out in the following manner. A small bit of the material was transferred to a tube containing 5 cc. of brilliant green broth (brilliant green 1:200,000 in broth of pH 7.4) and crushed inside the tube. To the tube there was then added 0.25 cc. of a 1 per cent sterile aqueous solution of lead acetate. The tube was incubated at 37°C. over night. If on the following morning the precipitate in the tube was brown, showing evidence of sulfide production (presumptive test of the presence of *Bacillus typhi murium*), a loopful of the broth was smeared on lactose-saccharose-neutral red agar, pH 7.4, plates containing 1:400,000 brilliant green. Colonies from these

plates were fished into lead acetate agar tubes containing four sugars:[3] lactose, raffinose, saccharose, and salicin, 0.25 per cent of each. Cultures having the characteristic reactions of *Bacillus typhi murium* were then agglutinated with two monotypical immune sera. The longer incubation period for brilliant green broth tubes has revealed a higher percentage of deaths attributable to mouse typhoid than was obtained with the usual procedure of 1 hour's incubation in a water bath.[4] The addition of lead acetate to the broth greatly reduces the number of brilliant green plates required. The first few hundred examinations with this technique were controlled by the usual culture method resulting in a gain of about 10 per cent in the positive findings. The lead acetate did not fail in a single instance to reveal *Bacillus typhi murium*, and in 125 tubes, in which there was no browning controlled by plating, no *Bacillus typhi murium* was found. Many brilliant green tubes containing feces may show the sulfide reaction when no *Bacillus typhi murium* is present, due to the growth of other sulfide-producing organisms such as *Bacillus alkaligenes*, etc. The plating on brilliant green and transferring of the colonies to the four sugar tubes control this point.

The final agglutination test is highly important as endemic strains, differing antigenically from the strain used to induce the artificial infection, are sometimes found in mice succumbing in course of the epidemic experimentally set up.

[3] The composition of this medium is as follows:

Beef extract	3 gm.
Peptone	10 "
NaCl	5 "
Agar	15 "
Lactose	0.25 per cent.
Raffinose	0.25 " "
Saccharose	0.25 " "
Salicin	0.25 " "
Andrade indicator	1 " "
Distilled water	1,000 cc.

pH 7.2

Lead acetate ... 1 cc. of 0.25 per cent solution to each 4 cc. tube of medium.

[4] Undoubtedly in our earlier series many negative results were recorded which with the newer technique would have been found positive.

Bacillus typhi murium Employed.

A bacillus conforming to the cultural characteristics of *Bacillus typhi murium* and pathogenic for mice was isolated from an epizootic among a cancer breeding stock.[1] This strain (Mouse Typhoid I) was passed by intraperitoneal injection through 5 mice. A suspension containing 1:20 of an 18 hour culture on slant agar was then fed to a mouse, and on the death of this animal the bacillus recovered, after being identified culturally as belonging to the same group, was fed to 4 mice in succession. As it happened, later immunological tests showed the strain recovered from the final mouse of this series to be antigenically different from the original strain but having identical fermentative properties with those of so called *Bacillus typhi murium* and being pathogenic for mice. It is probable that this strain which we term Mouse Typhoid II was enzootic among the stock used in the preliminary experiments, replacing somewhere in the series of 4 mice the strain used originally to induce infection. It came out in later studies that the second large outbreak among the cancer breeding stock[1] was caused by a strain antigenically identical with this strain. However this may be, the strain acquired highly invasive powers, as the experiments to be described will show. From this point on, this strain appeared in the animals succumbing to the mouse typhoid arising in the course of our experiments except in a few instances in which an immunologically different strain was obtained in culture. The fact of this substitution of strains emphasizes the importance of making regular immunological tests on all strains employed in and recovered during the experiments.

In referring to this bacillus with which our experiments were conducted as *Bacillus typhi murium*, we would have it understood that it belongs to the class of bacilli embraced under this term and was later found to be indistinguishable by immunological tests from *Bacillus pestis caviæ* of the paratyphoid B group. The immunological reactions of the bacillus will be described in a separate communication.[5,6]

Preliminary Culture Feeding.

Feeding Series B.—November 2, 1919. 18 normal mice, after fasting 24 hours, were fed during 24 hours with milk containing 1:20 culture of Mouse Typhoid II

[5] Amoss, H. L., and Haselbauer, P. P., *J. Exp. Med.*, 1922, xxxvi, 107.
[6] Webster, L. T., *J. Exp. Med.*, 1922, xxxvi, 97.

from an 18 hour growth on slant agar. During the next 18 days, 15 died and yielded positive cultures of the same bacillus in the feces and spleen; in 8 the bacillus was recovered also from the gall bladder. The deaths took place as follows: 3 occurred in 7 days; 4 in 8 days; 2 in 9 days; 2 in 10 days; 1 each in 11, 15, 17, and 18 days. On the 36th day the 3 surviving mice were killed, of which 2 proved fecal carriers. The bacillus was not found in the spleen or gall bladder.

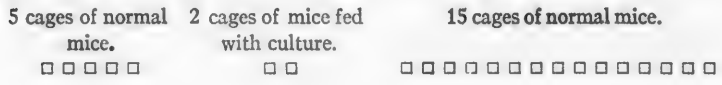
Series C.—The day after the feeding of the culture in milk just described, 13 mice (Series C) were placed in 2 cages of 5 and 8 respectively, and placed beside the cages containing the fed animals. They were all cared for by the same attendant. Of the cage of 5 mice, 1 only succumbed to mouse typhoid and that on the 12th day. Of the other cage, all 8 succumbed, on the 11th, 13th, 14th, 15th, 17th (2), 19th, and 25th days respectively. The unequal distribution of deaths in this series need not be considered now; but the main point is that already the culture Mouse Typhoid II had exhibited power to induce fatal infection through mediate contact as well as through direct feeding. Moreover, the contact mice succumbed in periods not exceeding those of certain of the fed mice.

Series D.—13 days after the feeding experiment of November 2, 24 mice in 8 cages (Series D) were placed beside the cages containing the 11 surviving mice of Series C. 11 died and 8 yielded positive cultures. At the expiration of 60 days, the remaining 13 were killed. 5 of them proved fecal carriers.

Experimental Epidemic.

The culture Mouse Typhoid II was now regarded as probably capable of producing mouse typhoid by ingestion in a large proportion of the fed mice and also of inducing that disease in exposed or contact mice not directly fed. Whether it possessed also the particular qualities which might be required in order that the spread from animal to animal should take place in the manner common in epidemics remained to be ascertained. An experiment to test this point was next designed.

Series E.—December 12, 1919. Food was withheld for 24 hours from 10 mice. Each mouse was then given milk to drink containing a heavy suspension of culture Mouse Typhoid II. It is estimated that each animal received approximately 1:20 of an 18 hour agar slant culture. At the end of 24 hours the mice were transferred to clean cages, 5 in each. With this experiment in view, 100 normal mice were assembled on December 8, into 20 cages, and arranged in positions indicated by the following diagram.



These mice had been free of fatalities. Just here it should be stated that the mice assembled for all the experiments were not only home-bred and of a stock free of disease, but when introduced into the experiment they were of an average weight of 13 gm. and age of 4 weeks.

The series of events were now as follows: Of the 5 mice in feeding cage 1, 2 died on the 8th, 1 each on the 13th, 14th, and 15th days. From all, cultures of Mouse Typhoid II were obtained. Of the 5 mice in feeding cage 2, 1 each died on the 12th, 13th, and 17th days. The 2 remaining mice were killed on the 60th day; cultures were negative.

Of the 100 mice in the 20 contact cages, the 1st animal died on the 15th day and the next on the 17th day. Within 60 days a total of 10 mice had succumbed. Of the 90 survivors, 7 (or 8 per cent) proved fecal carriers. Only 11 of the 20 cages showed infected mice as indicated either by death or carriage. The position of the mice, in respect to the purposely infected animals and the direction of the feeding or the cleaning of the cages, had no appreciable effect on the incidence of the infection. Obviously the attendant, in spite of more than ordinary precautions to keep his hands clean, early became contaminated and spread the contamination unwittingly and irregularly after the 1st day or two of his operations.

It is evident that what was produced in this experiment (Series E) was not an epidemic outbreak but rather a sporadic occurrence of mouse typhoid. But the impending conditions seem to have been significant. For on January 15, 1920, 79 normal mice in 17 cages (Series F) were assembled and placed on racks immediately next the cages of Series E on the 34th day after the beginning of that experiment. The effect was striking. No deaths occurred within the first 5 days, but there were 4 in the second 5 day period. The highest recorded were in the third 5 day period, and this wave quickly subsided. Two smaller waves appeared with their crests in the seventh and the ninth 5 day periods. The distribution of deaths in cages and periods is shown below.

Distribution of Deaths in Series F.

Days.	Deaths in cages.	Total No. of deaths in period.
1–4	0	0
5–9	Nos. 6, 13, 13, 16	4
10–14	" 1, 3, 4, 4, 5, 6, 7, 8, 9, 15	10
15–19	" 1, 2, 2, 4, 5, 6, 6, 10, 13	9
20–24	" 1, 4, 8, 11, 11	5
25–29	" 2, 3	2
30–34	" 2, 3, 11, 12, 15, 16, 17	7
35–39	" 2, 4, 13, 16	4
40–44	" 1, 8, 8, 8, 15	5
45–49	" 7, 13, 17	3
50–54	" 12, 14	2
55–65	" 13, 14	2

About 75 days after exposure the survivors of Series F, 24 in number, were killed and examined as carriers. 1 only was found. The following tabulation summarizes the results.

Total mortality	55, or	70 per cent.
Corrected "	41, "	52 " "
Cage attack rate	17, "	100 " "
Carriers among survivors	(1 in 24)	4 " "

A word of explanation is required regarding the terms total and corrected mortality. The latter refers to dead mice from which bacillus Mouse Typhoid II was recovered in cultures. The technique employed at this date was one in which the brilliant broth tubes were incubated for 1 hour before plating. Further studies showed that with this method many of these organisms are missed. This fact taken together with results obtained at a later date leads us to infer that the gross mortality figure as given is very nearly correct.

Thus it appears that once what may be termed sporadic cases of mouse typhoid are made to arise in a population previously free of this infection, the introduction of fresh, previously unexposed individuals may suffice, after a certain delayed or incubation period of 5 to 10 days, to bring about a sharp outbreak of cases which may reach epidemic proportions. In this particular instance every cage was attacked and the death rate very high.

Series G.—On February 13, 1920, or 29 days after the preceding or F series was started and at a time when the deaths among the latter had fallen, for the 5 days preceding, to 4, 48 normal gray tame mice of the average ages and sizes to the white mice employed up to this time, contained in 10 cages, were introduced into the mouse village and placed next the survivors of Series F. Instances of mouse typhoid arose among them and 25 mice died. On April 12, or 2 months after the placing of these mice in the village, the survivors were killed and examined for carriage of the bacillus Mouse Typhoid II. The following tabulation summarizes the results of this test.

Total mortality	25, or	52 per cent.
Corrected "	24, "	50 " "
Cage attack rate	10, "	100 " "
Carriers among survivors	(4 in 23)	17 " "

In brief, this series behaved very much as did the preceding one in respect to the several points covered in the study. It may be desirable

to point out the close correspondence between the total and corrected mortalities resulting from the improved bacteriological technique described on page 27.

It now became evident that it was possible to inaugurate epidemics of mouse typhoid among a healthy previously unexposed mouse population. Up to this time the events described are remarkably consistent. But as the experiment was continued by the successive introduction of fresh, vulnerable material, variations (or better stated perhaps wider fluctuations) made themselves apparent as we will now proceed to show.

At this point one or two incidental observations are called for. In the mouse room as arranged the racks carrying the cages were placed along the sides of two opposite walls, so that the cages could be made contiguous or could be separated by the distance of the width (12 feet) of the room. This separation did not in itself affect the closeness of contact, since in no instance did the mice actually mingle, but, as already stated, the intermediation of infected and non-infected animals was secured through the hands of the attendant.

However, the separation of cages by the width of the room made possible the carrying out of devices which might affect the mediation. For example, the attendant was made to use approved methods of hand sterilization[7] and to clean the cages and feed the mice on the side of the room away from the series in which the infection existed, before tending the latter.

Series H.—Thus on February 13, 1920, or the same day that Series G was brought into the village and placed in cages immediately adjoining those of Series F, 100 healthy mice were assembled in 20 cages (Series H) and placed by themselves on shelves along the opposite wall at a distance from those already in the room, where they were kept for 2 weeks. As deaths among these mice began within 2 weeks, it is evident that the carrying out of sterilization by the attendant did not suffice to render his hands free of contamination with the strain of bacillus employed in the infection experiments. The series was now brought over and placed next to Series I, described below. Deaths among them continued so that at the end of 2 months, April 12, the results were as follows:

[7] The cleaning of the hands of the attendant was carried out in the following order. 10 minutes scrubbing with hand brush, using tincture of green soap. Wash in warm tap water. 2 minutes in potassium permanganate solution. Then immerse in saturated solution of oxalic acid until stain is removed (3 to 5 minutes). Rinse in tap water.

Total mortality	33 per cent.
Corrected "	25 " "
Cage attack rate	(14 in 20) 70 " "
Carriers among survivors	(8 " 67) 12 " "

The lowered death rate and the reduced cage attack rate are at once apparent. That the events summarized in the tabulation accompanying Series H are not mere exceptions is shown by the next experiment.

Series I.—February 18, 1920. 177 healthy mice, distributed in 36 cages, were brought into the village and placed adjoining and in line with Series G. These healthy animals also were tended without sterilization of hands and tools, after Series G, in which the infection was still proceeding. The detached Series H was cared for first of all. It was supposed that should the infection appear among the mice of Series I, it would attain a degree of activity capable of being accelerated by the available mass of new infectible material introduced at about the period of the preliminary outburst, while the mice in the detached Series H might acquire some resistance due to slow infiltration of virus. As a matter of fact, the two series, H and I, behaved quite as if they had been one series and all the mice had been brought into the village at one time. The experiment involving Series I was terminated on May 18, or after 3 months. The tabulation gives the final outcome.

Total mortality	64, or 36 per cent.
Corrected "	47, " 26 " "
Cage attack rate	(28 in 36) 80 " "
Carriers among survivors	(14 " 113) 12 " "

Review.

It is not our intention to enter into a minute discussion in this place of the significance of these experimental data. Such discussion as we purpose to give the subject will be presented in connection with the next paper in which the succession of events taking place day by day in the several series will be described.[8] We prefer merely to pass in review in this place the salient facts connected with the experiments detailed.

The feeding of the mice exposed in the village and the cleaning of the cages were done by one person and always in the same direction.

[8] Amoss, H. L., *J. Exp. Med.*, 1922, xxxvi, 45.

As each new series was introduced, its cages were first tended. Series G and I were brought into the mouse village when the epidemic in Series F was at the crest of the wave. Series F (79 mice) was assembled and exposed on January 15, Series G (48 mice) on February 13, Series H (100 mice at distance until February 27) on February 13, Series I (177 mice) on February 18. Series F, G, and I were contiguous; Series H was separated by the distance (width) of the room (12 feet). Before the cages of Series H were touched, the attendant scrubbed his hands and nails with soap and brush for 10 minutes and used permanganate of potash and oxalic acid as disinfecting solutions. After 2 weeks separation Series H was brought into contact with Series I.

The several series may be regarded as having been placed in a single line, with the cleaning and feeding carried out in one direction. Thus following Series F there are 10 cages of Series G containing 5 mice each, followed by 36 cages of Series I and 20 cages of Series H, also of 5 mice each. Numbering the cages in order, Series G would be covered by cages 1 to 10; Series I by cages 11 to 46; and Series H by cages 47 to 66 inclusive. It might be expected that mice would be first attacked and die in cages 1 to 5 and that the infection would spread by contiguity. But the order is not so regular as this and the contrary may happen, for the first mice to die of mouse typhoid were in cage 7; then in cages 2, 3, and 4; then in Nos. 2, 3, and 5; then in Nos. 9 and 10. In Series I, of 36 cages, exposed 5 days after Series G, the distribution was as follows:

Days.	Deaths in cages.
1–4	Nos. 20, 29
5–9	" 11, 16, 17, 23, 25, 34
10–14	" 18, 24, 27, 28, 33, 34, 39
15–19	" 11, 13, 14
20–24	" 13, 23, 30, 32, 34
25–29	" 13, 26, 32, 33
30–34	No. 30
35–39	Nos. 26, 27
40–44	" 24, 29
45–59	" 11, 19, 21, 24

The one safe deduction from this tabulation seems to be that a wide but not uniform distribution of the bacillus is quickly brought about by the attendant through which individual mice, in an entirely unpredictable order, take it up and fall victims to the infection ensuing. Once this has happened the cages must soon become contaminated widely by the excrement carrying the bacillus and all the remaining mice should receive the organism. The sources of the subsequent irregular events can only be inferred as depending upon such factors as dosage and possibly fluctuations in pathogenic activity of the bacillus, and upon variations in the resisting powers of the mice. These factors are the ones commonly invoked to explain such vagaries of case incidence of the communicable diseases as here presented and are set down here not as finalities or even as matters for discussion, but rather to emphasize a parallelism existing between the natural, so called, infections in man and animals and those purposely set up, as in the instance being considered, which may be used in putting the next question to be answered by experiment.

In concluding this presentation, a table has been prepared of the five series of mice exposed to infection with the bacillus of mouse typhoid (Table I). The number of mice in each series varied from 48 to 177. In the table, Series I, which contained the largest number given, is broken up into three parts called first, middle, and last, according to the degree of removal of the cages at the time they were brought into the mouse village from the mice which were already potentially infected. It is not suggested that the factor of nearness or remoteness is a controlling one, but the division is interesting as bringing out again the element in the process of distribution of the infection which for the present is merely termed vagary. It is to be kept in mind that the quality of mice in the three divisions was superficially homogeneous. The only mice fed with culture were 10 of Series E. The rest of the animals received the bacillus through the exigencies of contact between them, their habitation, and the hands of the attendant.

The figures in Table I not only speak for themselves but have already been discussed in the earlier pages of this paper. The exception is Series I in which the three groups of approximately 60 mice each, selected by degree of removal from the older infected series into

relation with which they were brought, show a mortality of 38, 56, and 15 per cent respectively. Since the first group of 60, immediately next the older and already infected series, gave a lower mortality than the middle and further removed group, the very low mortality in the last group can hardly be accounted for by position alone.

TABLE I.

Composite Table of the Five Series.

Series.	No. of mice in series.	Mortality.	Corrected mortality.	Carrier rate among survivors.	Carrier rate in total No.	Cage attack rate.
		per cent	*per cent*	*per cent*	*per cent*	*per cent*
E*	100	10	10	8	7	55
F	85	70	52	4	1	100
G	48	52	50	17	8	100
I First	60	38	22	11	7	83
I Middle	60	56	52	4	2	100
I Last	57	15	5	18	15	50
H	100	33	25	15	10	70

* The infection in this series was produced by feeding with a suspension of *B. typhi murium* 10 of the mice.

The tabulation brings out a striking irregularity between carrier and death rate. Thus of 14 carriers, 8 were found in the same number (8) of cages in which no deaths from mouse typhoid took place, while 6 were found in 29 cages in which deaths from the infection took place. The distribution of carriers according to deaths in the cages is as follows:

No. of deaths in cage.	Instances of carriage of bacillus.
0	8
1	3
2	1
3	2
4	0

Experiments with Strains Arising from a Single Bacillus.

Six strains were obtained from single cells of Mouse Typhoid II by Barber's method. In order to be certain that only single cells

were picked, the strains obtained were plated, a colony was picked, and from the 8 hour growth in broth a single cell was again isolated and the same process repeated. Thus before a strain was considered as arising from a single cell, the culture was analyzed by the Barber method three times.

Six such pure-line strains were obtained, three of which showed slightly greater virulence by intraperitoneal injection into mice, as shown in Table II.

TABLE II.

Results of Intraperitoneal Injection of 1:30,000 of a Culture of Each of Six Single Cell Strains of Mouse Typhoid II.*

Weight of mice.	Length of life.					
	Strain.					
	A	D	E	F	G	H
gm.	*hrs.*	*hrs.*	*hrs.*	*hrs.*	*hrs.*	*hrs.*
13	52	44	66	20	66	64
	60	60	78	54	72	66
14	75	78	100	64	84	72
	88	78	105	66	90	78
15	80	82	115	72	94	100
	90	86	134	78	134	138
16	96	94	150	84	150	200
	111	110	160	99	200	255
17	130	130	255	115	408	260
	140	132	S.†	200	520	495

* The suspensions were standardized in a turbidimeter.

† S. indicates survived.

Comparison of the Virulence of Single Strain A with the Composite or Original Strain of Mouse Typhoid II.—Since the results of intraperitoneal injections (virulence tests) of mice with the mouse typhoid organisms vary considerably, it becomes necessary to use large numbers of mice in each experiment. With the view of determining what mice grouped according to weight would yield the most concordant results, the following experiment was made.

Experiment 1.—Mice were selected from a large stock until 10 of each weight from 10 to 17 gm. were obtained. Three lots of 6 each, composed of individuals weighing 18, 19, and 20, and 1 weighing 21 gm., were selected. Each of the 99 mice received intraperitoneally in 1 cc. of salt solution 1:30,000 of a 16 hour slant agar culture of Mouse Typhoid II. This strain had been plated repeatedly so that the culture represents the descendants of a single colony.

The number of hours before death is recorded for each mouse in Table III.

TABLE III.

Relation of Weight to Susceptibility to Intraperitoneal Injection of Mouse Typhoid, Strain II.

November 30, 1920. Each mouse received intraperitoneally in 1 cc. 1:30,000 of a 16 hour growth on pH 7.4 agar; suspension standardized in a turbidimeter.

Length of life.											
Weight of mice.											
10–10.5 gm.	11–11.5 gm.	12–12.5 gm.	13–13.5 gm.	14–14.5 gm.	15–15.5 gm.	16–16.5 gm.	17–17.5 gm.	18–18.5 gm.	19–19.5 gm.	20–20.5 gm.	21–21.5 gm.
No. of mice.											
10	10	10	10	10	10	10	10	6	6	6	1
hrs.	*hrs.*	*hrs.*	*hrs.*	*hrs.*	*hrs.*	*hrs.*	*hrs.*	*hrs.*	*hrs.*	*hrs.*	*hrs.*
20	20	20	20	49	20	20	20	46	20	20	89
20	20	27	25	66	30	59	20	73	60	20	
25	20	28	40	72	52	64	20	86	70	30	
48	41	60	50	75	52	86	27	106	75	60	
50	63	60	65	78	60	89	30	115	91	67	
69	67	72	74	79	73	90	34	130	96	75	
86	67	83	75	86	90	118	75				
98	67	86	89	115	115	118	86				
106	86	93	93	125	130	118	88				
110	113	104	106	160	144	160	90				

The number of deaths recorded in each group at 50, 75, 100, and 150 hours shows little correspondence, so that no conclusion can be reached in the matter of the selection of the most suitable group for testing virulence. If, however, a single cell strain of this micro-organism is employed, there appears in the same kind of experiment a definite relation between the susceptibility and age groups, as is shown in Experiment 2.

Experiment 2.—120 mice were selected so that there were groups of 10 of each weight from 10 to 21 gm. Such mice received intraperitoneally in 1 cc. 1: 30,000 of a 16 hour slant agar growth of single cell strain Mouse Typhoid II A. The suspensions were standardized in a turbidimeter to the density of the suspension used in Experiment 1. The results are shown in Table IV.

TABLE IV.

Relation of Weight to Susceptibility to Intraperitoneal Injection of Single Cell Strain Mouse Typhoid II A.

January 13, 1921. Each mouse received intraperitoneally in 1 cc. 1:30,000 of a 16 hour growth on pH 7.4 agar; suspension standardized in a turbidimeter.

Length of life.											
Weight of mice.											
10–10.5 gm.	11–11.5 gm.	12–12.5 gm.	13–13.5 gm.	14–14.5 gm.	15–15.5 gm.	16–16.5 gm.	17–17.5 gm.	18–18.5 gm.	19–19.5 gm.	20–20.5 gm.	21–21.5 gm.
No. of mice.											
10	10	10	10	10	10	10	10	10	10	10	10
hrs.	*hrs.*	*hrs.*	*hrs.*	*hrs.*	*hrs.*	*hrs.*	*hrs.*	*hrs.*	*hrs.*	*hrs.*	*hrs.*
50	20	26	14	20	20	40	70	16	18	96	90
58	20	28	16	60	58	58	86	75	90	124	117
64	22	58	52	67	60	64	88	90	96	136	168
66	26	68	70	70	74	70	90	106	132	150	168
70	34	70	74	85	75	80	111	117	134	188	186
74	42	74	106	100	88	90	132	142	158	202	206
85	52	90	114	132	148	124	130	184	266	248	208
90	100	96	122	132	168	140	140	230	304	260	260
154	108	108	140	150	254	312	250	264	308	296	404
158	115	115	180	163	256	S.	266	S.	624	404	630

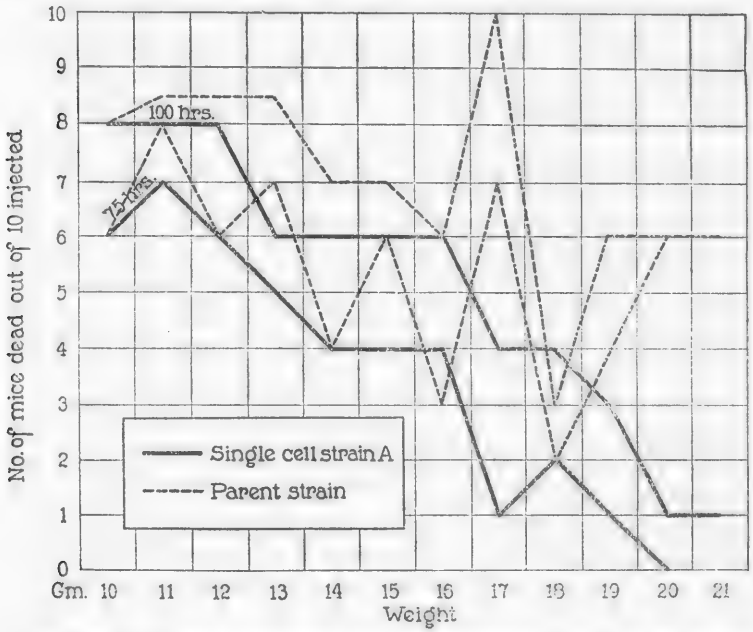
A comparison of the isolethal lines at 75 and 100 hours in Experiments 1 and 2 is shown graphically in Text-Fig. 1.

Of the two, the parent strain seems to be more virulent. The irregularity of the results in Experiment 1 and the obvious correlation between body weight and susceptibility shown in Experiment 2 with the single cell strain point to individual variation among the microorganisms comprising the parent strain.

Pathogenicity of Single Cell Strains Administered by Mouth. Experiment 3.—The power to infect by mouth of three of the single cell strains was tested by allowing groups of 8 mice each to drink milk containing living cultures of the strain to be

tested. Of 8 mice receiving Strain A, 5 died and 3 were living after 30 days; with Strain F 6 mice died and with Strain H only 2 died. Strain F was therefore selected in the experiment to be described, in which attempts were made to start an epidemic of mouse typhoid under the conditions known to be favorable.

Series O. Experiment 4.—February 10, 1921. Each of 10 normal mice from which food had been withheld for 24 hours was fed by stomach tube 1:250 of a 16 hour slant agar growth of pure-line Strain F.



TEXT-FIG. 1. Comparison of the relation of the virulence of a single cell strain and of the parent strain to body weight of mice. The injections were made intraperitoneally. The difference in weight of the 10 mice in a given group was not more than 0.5 gm.

February 11. The 10 mice in 2 cages, 5 in each, were placed midway on a shelf among 20 other cages, each containing 5 normal mice. During the following 46 days, 6 of the feeders and 7 of the contacts died of mouse typhoid.

On the 46th day of observation 100 normal mice in 20 cages (Series P) were brought into line with Series O. There was no sharp increase in deaths among the normal mice added or among the original. Thus among the new mice (Series P) the first death from mouse typhoid occurred 3 days later, and one on the 14th,

18th, and 36th days. Within 98 days only 11 of the mice died. During this period 10 of Series O died of mouse typhoid, making a total of 17 of the contacts in Series O and 6 of the feeders.

Since the addition of fresh normal mice in the above experiment with pure-line Strain F did not result in an outbreak of mouse typhoid under conditions which in our experience constantly incite epidemics with Mouse Typhoid II, another pure-line strain was employed in the next experiment under slightly different conditions; *viz.*, the new mice were added on the 31st day as in Series K, L, and M[9] instead of the 46th day as in Series O and P.

Series Q. Experiment 5.—June 8, 1921. Each of 10 normal mice from which food had been withheld for 24 hours was allowed to drink milk containing 1:20 of a 16 hour agar slant growth of pure-line Strain A, isolated from Mouse Typhoid II and belonging to the three more virulent pure-line strains.

On the following day the mice were placed in 2 cages, 5 in each, situated midway in a line of 20 cages, each containing 5 normal mice. During the following 30 days only 2 of the contact mice died. None of the feeders succumbed. On the 31st day, 100 normal mice in 20 cages (Series R) were added in line with Series Q. During the next 112 days only 7 of these mice died.

Comment.—Pure-line Strains F and A came from single cells picked from Mouse Typhoid II, the strain which was used to start the long replacement series, K, L, and M. It will be seen in another paper in this issue[8] that in the latter series the addition of new normal mice during a quiescent period was followed shortly by a new outbreak, first among the new or added mice and then among the old mice; that is, mice which had been for some time exposed to the virus. Under these same conditions of feeding, arrangement, and time under which the epidemic waves in Series K, L, and M were established with Mouse Typhoid II, the pure-line strains signally failed to induce an epidemic. Thus the individuals from which Strains F and A descended lacked the power to produce an epidemic under the same conditions which sufficed for the composite strain Mouse Typhoid II to incite an epidemic outbreak.

[9] These series are described at length on p. 46 of this issue.

SUMMARY.

In this paper we have described the first part of an experimental study of the epidemiology of mouse typhoid. One set of data has been presented on the basis of which little or no analysis has been attempted. The immediate object has been rather to collect materials than to undertake to account for the phenomena encountered. It is obvious that the factors involved in the inquiry are intricate, but it is believed that they are not necessarily or all beyond disentanglement. About 500 mice in all have been studied in an experimental village, brought together in increments among a population in which mouse typhoid experimentally induced was prevailing.

The results have been presented according to two phenomena; namely, mortality or death rate, and bacillus carriage rate. The material does not lend itself to consideration according to morbidity rates. If it were established that every instance of attack, when not fatal, was attended by carrier production for the bacillus of mouse typhoid, reliable morbidity tables could be constructed. In the absence of this certain criterion, the materials here presented can be dealt with only as mortality data. This fact is attended with obvious disadvantages in respect to the epidemiological material assembled regarding infectious disease in man. In spite, however, of the drawbacks, it is already evident that the results obtained by the sort of inquiry here described may come to throw no inconsiderable light on moot problems on the origin, mode of spread, and manner of decline of epidemic diseases in general.

The analysis of the strains by selecting single cells and thus establishing substrains has yielded results which may eventually have value in explaining fluctuations in virulence. Among the positive data arising from the experiments with such cultures are, first, that there have been obtained by mechanical means from the ordinary bacteriologically pure culture, single cell strains exhibiting slightly different pathogenic activity, whether administered by mouth or parenterally, and second, that more regular results are obtained with intraperitoneal injections of these strains than with the parent strain. Among the negative results to be recorded are the failures of two single cell strains to incite an epidemic among mice under conditions known to be suitable when the parent strain is used.

EXPERIMENTAL EPIDEMIOLOGY.

II. Effect of the Addition of Healthy Mice to a Population Suffering from Mouse Typhoid.

By HAROLD L. AMOSS, M.D.

(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

(Received for publication, April 6, 1922.)

In earlier papers of this series an ordinary or spontaneous epidemic[1] of mouse typhoid and an artificially induced outbreak[2] of the same disease have been described. The purpose of the present paper[3] is to consider more minutely the effect of bringing a healthy stock of mice into a community in which mouse typhoid is prevailing. The essential fact that such an addition of healthy mice leads to the transfer of the infection from the old population to the new comers, and the gross mortality figures thus resulting[2] have been pointed out. But the manner of the spread of the disease from the old to the new, and the reaction or effect of the revived epidemic on the old and previously surviving individuals, need to be dealt with in detail.

Method.

Three series of 100 mice each were assembled separately. The first, Series K, was used to start the outbreak by placing in it 10 mice which had been fed with living cultures, while the other two, Series L and M, were isolated to be brought 30 days later into contiguity with the first. After the three series had been brought together the infection spread, resulting in an outbreak which then subsided to a low ebb in which no deaths occurred for 1 week. Other new healthy mice were then introduced to bring the total number up to the original strength.

[1] Lynch, C. J., *J. Exp. Med.*, 1922, xxxvi, 15.

[2] Amoss, H. L., *J. Exp. Med.*, 1922, xxxvi, 25.

[3] An abstract of this paper was presented at the 36th Session of the Association of American Physicians, Atlantic City, May, 1921 (Flexner, S., and Amoss, H. L., *Tr. Assn. Am. Phys.*, 1921, xxxvi, 34).

After the new wave thus excited had receded, it became the rule to recruit again, by the introduction of healthy mice. Twelve such replacements were made during the period covered by this report.

EXPERIMENTAL.

Exciting the Epidemic.

Experiment 1.—Series K: May 1, 1920. 100 healthy mice were assembled in 20 cages of 5 each. May 3. 1 mouse found dead; no *B. typhi murium* detected. May 13. Second mouse found dead and *B. typhi murium* isolated from feces.[4] May 20. As no further deaths occurred, there were placed, midway in the series, 2 cages of 5 mice each, which had been fed during 24 hours on milk containing a suspension of *B. typhi murium*. It is estimated that each of the 10 mice received about 1:20 of an 18 hour agar slant culture. During the next 23 days 12 deaths occurred from mouse typhoid, 6 among the 10 fed and 6 among the 100 contact mice. The last of these deaths occurred on June 21, on which date Series L and M were brought into the room containing the infected Series K.

Series L: 100 mice had been assembled also on May 1. Between this date and June 21, 3 of the mice had died, and *B. typhi murium*[5] had been recovered from the spleen and feces in each instance.[4] On June 21 the 20 cages of Series L were placed in a row immediately adjoining those of Series K.

Series M: This series,[6] numbering 100 mice, had also been assembled on May 1. Between that date and June 21, when the 20 cages were brought into the room with infected Series K and placed immediately next the cages of Series L, 3 mice had died, and *B. typhi murium*[5] had been obtained from the spleen and feces of each.[4]

It is shown in Text-fig. 1 that the fatalities in Series K resulting from contact with the fed mice were very low—6 per cent. With-

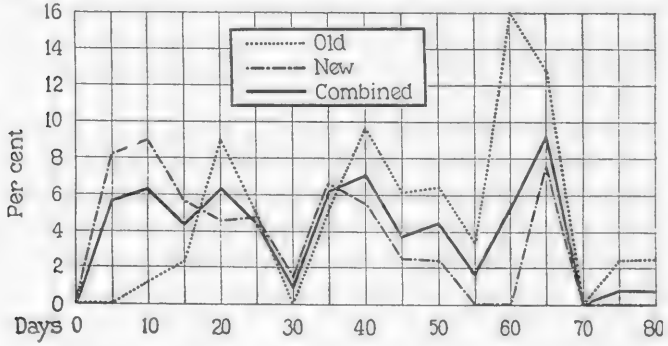
[4] Cultures from the spleen and gall bladder were negative. Three deaths from mouse typhoid occurred in each of the other two series, L and M, before exposure to the experimental infection. All came from the same stock of about 3,000 mice among which there occurred 6 deaths from mouse typhoid during the spring of 1920. It is presumed that in assembling the series one or more carriers was included. However, the series were for this reason kept isolated for 20 to 30 days before the experiment was begun.

[5] At this time the differences in Mouse Typhoid I and Mouse Typhoid II were not known.

[6] Series L and M were identical. The former was contiguous with Series K and the latter 20 cages removed from Series K, but contiguous with Series L. These two series are kept separate in order to observe the effect of distance from the source of infection on the manner of spread, and also for convenience in keeping records.

in 5 days after Series L and M were brought in, 6 deaths occurred in Series L and 9 in Series M, a total of 15; and in the next 5 day period a total of 19 deaths occurred in the two series. No deaths occurred in Series K during the first 5 day period and only 1 death in the second 5 day period.

The immediate result, then, of the introduction of normal animals was an outbreak among those mice brought in, whereas deaths in Series K did not increase until during the third 5 day period. The epidemic progressed in a succession of waves in each of the three series. In the like Series L and M, the waves were synchronous throughout the period, and the crests were lower and the intervals between greater



TEXT-FIG. 1. Death rate by 5 day periods in Series K, combined death rate for Series L and M, and combined rate for the three series, during the first epidemic period.

as the epidemic proceeded. The wave in Series K lagged behind Series L and M, and in contrast rose higher and the intervals between them became shorter. The lag of Series K decreased until at the twelfth period the wave of this series was coincident with the smaller Series L and M waves.

The three series were kept intact and without increments for 70 days after the normal mice had been brought into contact with Series K, at which time the epidemic had reached a low ebb. The total mortality among the approximately 300 mice in the entire period of 85 days after the introduction of the new mice was 54 per cent; in Series K 62 per cent (56 out of 91); in Series L 40 per cent (38 out of 97); and in Series M 61 per cent (59 out of 97).

The mortality among the added Series L and M combined was 50 per cent, while for the same period, the mortality in Series K (exposed from the first to the culture-fed group) was 62 per cent.

Comment.

The conditions of the experiment involving Series K, L, and M were similar to those described in the first paper of this series, in which first a sporadic outbreak of mouse typhoid was started by bringing a small number of mice purposely fed with a culture of *Bacillus typhi murium* into an assemblage of healthy mice; and the sporadic incidence was later fanned into an epidemic outbreak by the introduction of fresh groups of healthy mice at a time at which the occasional deaths had practically ceased. Hence the events recorded in the former paper seem not to have been accidental in nature but rather to follow a certain rule.

The course of the fatalities in the K, L, and M series is a definite one. In the first place the distribution of deaths in the cages was such as to point, just as in the earlier experiments, to an early general dissemination of the bacillus. Thus the first fatalities occurred in cages remote from those of the fed mice. But more significant is the fact that once the infection appeared among the mice of the L and M series, the number of fatalities not only rose steadily to a maximum, but was reflected backward to the K series in which no further deaths were taking place at the time. The precise manner of the rise in the L and M series, the exact period of delay in the K series induced by the occurrences in the former series, and the final result, or sum total of fatalities in each series, are shown graphically in Text-fig. 1.

To undertake to account for these happenings is to enter, at the moment, the realm of speculation. Two possible explanations present themselves. According to one it may be supposed that the bacillus of mouse typhoid was whipped into an exalted virulence by being rapidly passed from one susceptible mouse to another until this was so great as not only to reach a degree of infectivity greatly augmented for the new mice of Series L and M, but also to increase its striking power for the mice of Series K which, for a 30 day period, had successfully weathered exposure to the purposely fed mice introduced into the midst of that group.

According to the other supposition the effects described do not depend primarily on augmentation of pathogenicity but rather on increase in mass of the bacillus and result not from virulence so much, or alone, as from dosage. This view involves the conception that favorable conditions of growth and multiplication of the bacilli in the exposed mice are the more decisive events. It would seem as if these favoring circumstances did not exist in the first series employed but did arise subsequently among the new exposures. Once, however, they were secured, the growth and multiplication became such as to overwhelm not only the new series but also the older, previously exposed, survivors, among which the death rate was finally greater than among the new.

Probably the precise differences in the percentage mortalities are not significant. Series L and M were practically homogeneous and yet the one (L) gave a mortality of 40, and the other (M) of 61 per cent, the latter agreeing almost exactly with that of the K series. Moreover, according to this comparison, there are no grounds for supposing that the survivors of Series K by merely having been previously exposed were better able to withstand the violence of a real epidemic than were the previously unexposed mice of Series L and M.

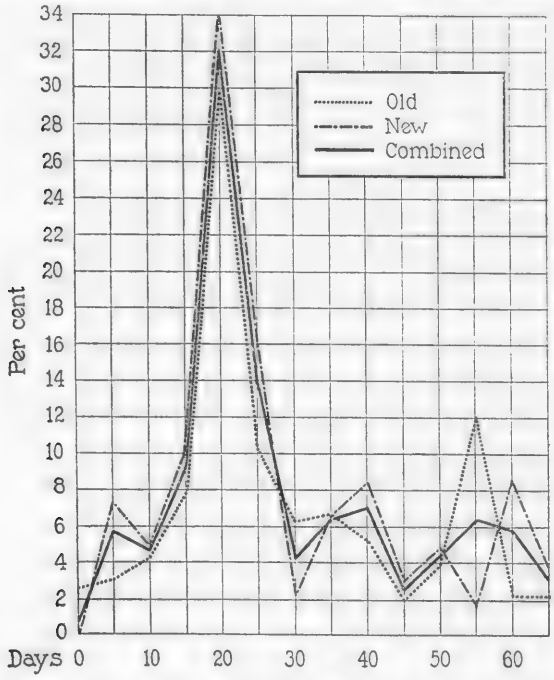
Hence it may be that the two factors mentioned, namely virulence and mass, are not only of high importance in themselves but also subject to such a degree of accurate measurement as to determine the potentially effective degree of each. All these considerations leave out of immediate account variation in the host—a factor at present not to be dealt with.

Effect of Replacements.

First Replacement, or R 1. Experiment 2.—September 9, 1920. The survivors of Series K, L, and M were observed during a period of 115 days at the expiration of which deaths took place seldom; during the final period of 7 days, no deaths at all occurred. At this juncture new mice from a healthy stock[7] were added to each cage in numbers replacing the deaths, so that each cage again contained 5 mice and the total population in the room was recruited to 300. In order to

[7] Among the stock of about 3,000 mice from which these mice were taken, there occurred 5 deaths from mouse typhoid in 6 months.

prevent losses from fighting between the old and the new mice, the latter were separated from the former by placing a coarse wire screen diagonally in the cage, separating it roughly into two compartments. While the mice themselves were thus grossly kept apart, yet they and their excrement came into contact.



TEXT-FIG. 2. Death rate by 5 day periods among the old and new mice, and combined rate, during the first replacement period.

The constitution of the several series as recruited was as follows: Series K now consisted of 98 mice,[8] of which 38 were old and 60 new; Series L of 100, of which 57 were old and 43 new; Series M of 100, of which 27 were old and 73 new. Hence the relation of old or exposed to new or unexposed mice totalled 122 : 176.

The new events came quickly. Within 5 days 17 deaths occurred, 13 among the new and 4 among the old mice. In the next 5 days

[8] This series was recruited to a total of 100 mice but 2 were killed in fighting.

there were 13 deaths, 8 among the new and 5 among the old. In the third 5 day period the deaths numbered 25, 16 of which were among the new and 9 among the old animals; while in the fourth 5 day period (20 days after the admixture of old and new mice) 79 deaths occurred, 48 of which were of new and 31 of old. This constituted the peak of the epidemic, after which the death rate fell off sharply.

These data are presented graphically in Text-fig. 2, from which it will be observed that the outbreak proceeded in two quite distinct waves among the new mice and in a steadily increasing progression among the old mice. This replacement, afterwards referred to as R 1, was observed for 65 days, during which a gross mortality from mouse typhoid of 69 per cent, made up of mortality K 86 per cent, L 68 per cent, and M 52 per cent, was noted.

Agglutinins in the Serum of the Survivors.

The serum of 56 of the survivors of the first replacement series was tested[9] for agglutinin with Mouse Typhoid II in dilution from 1:20 to 1:160. The serum from 37, or 66 per cent, partially agglutinated the strain in a dilution of 1:40, and of these, 20 showed complete agglutination in 1:40 and 2 showed complete agglutination in 1:160. The serum of 27 of the 56 mice showed no agglutinin in 1:20. Further analysis of the results shows that the serum of the 20 mice agglutinated the strain completely in a dilution of 1:40,

[9] The blood was collected from the mice by bleeding from the end of the tail. The mouse to be bled was placed in a small mailing case, one end of which was perforated and a single hole made in the other cap for the protrusion of the tail. The case containing the mouse was held in a clamp over a microscope lamp, the heat from which caused increased blood flow to the tail vessels. The tail was wiped with gauze moistened with 70 per cent alcohol, and after snipping with sharp scissors the drops of blood were allowed to flow by capillarity into a small bore, 1 cc. pipette graduated in hundredths. When 0.05 cc. had been collected, the end of the pipette was placed in isotonic salt solution and suction applied with a syringe until the mixture attained a total volume of 0.5 cc. The mixture now containing 10 per cent blood was delivered immediately into a small agglutination tube, mixed thoroughly, and allowed to clot. The following morning the clear fluid containing serum 1:20 was pipetted off with a small bore pipette, a syringe being used for suction.

while in 36 agglutination was either partial in 1:40 or absent. Of the former only 1 mouse, and of the latter 8 mice died during the second replacement series. Thus the death rate among the mice giving slight or no evidence of blood agglutinins was four times that noted among those possessing blood agglutinins.

Urinary Carriers.

Cultures of the urine of the same 56 mice were made on five occasions from November 15, 1920, to January 17, 1921. 35, or 62 per cent, were positive one or more times; of these 6 were positive during the entire period of 63 days. The serum of 5 of the 6 persistent urinary carriers agglutinated Mouse Typhoid II.

The results of the urine cultures and agglutination tests on the serum of the 56 mice are shown in Table I.

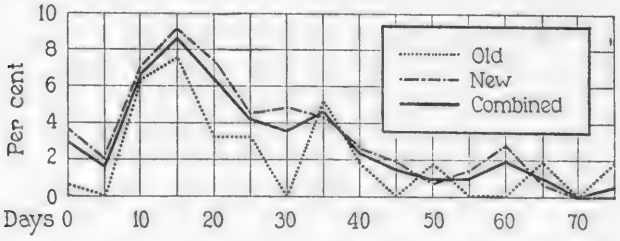
TABLE I.

Urine Cultures and Serum Agglutinins in 56 Survivors of the First Replacement Series.

	No.	Per cent.
Both agglutination and urine cultures positive	25	45
Agglutination positive, " " negative	12	21
No agglutination, urine cultures positive	10	18
" " " " negative	6	11

With these data before us we may now view the relative responses of the old or previously exposed and the new or not previously exposed mice to the impending infection. The number of old survivors which had already passed through an epidemic enduring 85 days and of which the total mortality was 58 per cent was 122. The death rate among these surviving animals induced by the new epidemic was 66 per cent, while the death rate among the 178 new or previously unexposed mice was 71 per cent. The percentage difference is too small to be significant, hence in all respects but one, namely that the new mice began to succumb before the first of the old, the two groups behaved in an identical manner. It is obvious that in the course of the second epidemic wave the gross mortality among the older mice exceeded that of the first epidemic, the proportions being as 58:71.

Second Replacement, or R 2. Experiment 3.—November 9, 1920. When the effects of the first replacement had subsided and no deaths at all occurred in a 5 day period, new mice, previously unexposed, were brought in as in the first replacement, to recruit the survivors in the three series to a total strength of 300. This second replacement was made 60 days after the first. The actual number of new mice introduced was 228, distributed as follows: Series K 87, Series L 82, Series M 59. Hence the proportion of old survivors to new population was as 72:228.



TEXT-FIG. 3. Death rate by 5 day periods among the old and new mice, and combined rate, during the second replacement period.

Text-fig. 3 shows that the new or R 2 mice began to die within 5 days, and 5 days later, or 10 days after the beginning of the experiment, deaths occurred among the survivors of the preceding epidemic. The experiment was allowed to run 75 days, at the expiration of which a total of 113 mice, or 38 per cent, had succumbed. The mortality in Series K was 29, L 40, and M 44 per cent, and among the old and the new mice it was 37.5 and 37.7 per cent, or virtual identity. That the old mice possessed no advantages over the new is further indicated by the patent fact that the number of the new (228) was approximately three times that of the old (72), thus increasing the probability of higher attack rate among the former. Ultimately the factors of age and size of the host may call for consideration. In those respects the surviving old differed materially from the younger and smaller new animals.

Third Replacement, or R 3. Experiment 4.—January 20, 1921. The third replacement of 114 new mice to recruit the new series up to 300 was made 75 days after the second. The series additions were almost equal: 66 in K, 65 in L, and 55 in M. During the next 60 days the total mortality was 14.3 per cent, distributed as regards old and new mice in proportion of 14 (old) to 14.9 (new) per cent.

This experiment was permitted to continue without interruption or modification for a period of 95 days, and it is worth while following the particular events which transpired. But first a few words are needed about the nature of the population composing the experiment. As is evident, the population is made up of mice of very different grades of exposure to mouse typhoid, since there have accumulated within it all the survivors of previous epidemics extending from May 20, 1920, of the first lot exposed, to January 20, 1921, when the third replacement was made. As Table II shows, the number of survivors of each replacement grows progressively smaller with each succeeding epidemic. Thus at the time of the third replacement period, what may be termed the combustible material consisted largely

TABLE II.

Distribution of Deaths According to Period of Introduction during the First 60 Days of the Third Replacement Period.

	Period of introduction of mice.			
	First epidemic (R).	First replacement (R 1).	Second replacement (R 2).	Third replacement (R 3).
No. of mice present	14	30	142	114
Deaths during first 60 days of Period R 3	1	2	21	17
Death rate, *per cent*	7	7	15	15
Survivors at 60th day of Period R 3	13	28	121	97

of the mice of the R 2 period. At the beginning of the R 3 period there were 14 mice remaining from the R period (first epidemic period), 30 from the R 1 period (first replacement), and 142 from the R 2 period. Reference to Table I shows that the mortality rate among the mice remaining from the R 2 period and the new mice was the same (15 per cent in each instance) and twice the rate observed (7 per cent) among the mice remaining from the R and R 1 periods.

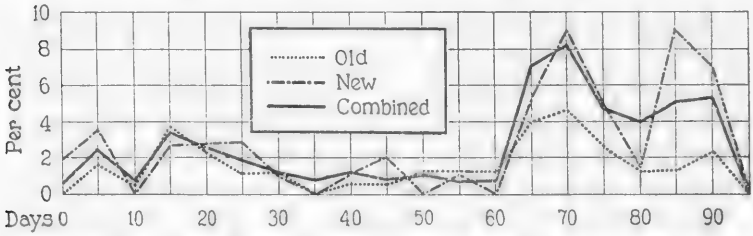
The events referred to may be summarized as follows: From the 25th to the 60th day, only an occasional death occurred in any of the series; for example, in Series K no death for 18 days; in Series L none for 29 days; in Series M none for 10 days. But at about the 60th day the deaths had reached epidemic porportions. This wave,

as it may be termed, was not induced intentionally but arose in the manner of similar waves in so called spontaneous epidemic outbreaks. Its duration was about 30 days, during which 73 (28 per cent) of 259 surviving mice after the first effect of the replacement subsided had succumbed. The distribution of deaths during this time, according to the period at which the mice were introduced, is given

TABLE III.

Distribution of Deaths According to Period of Introduction from the 60th to the 96th Days of the Third Replacement Period.

	Period of introduction of mice.			
	First epidemic (R).	First replacement (R 1).	Second replacement (R 2).	Third replacement (R 3).
No. of mice present at 60th day	13	28	121	97
Deaths from 60th to 96th days	0	4	35	34
Death rate, *per cent*	0	14	29	35
Survivors	13	24	86	63



TEXT-FIG. 4. Death rate by 5 day periods among the old and new mice, and combined rate, during the third replacement period.

in Table III. The death rate varied roughly inversely as the length of time the mice had been previously exposed. This higher mortality of 28 per cent is to be contrasted with the lower 14 per cent induced immediately by the repacement. The total mortality arising during the 96 days of the continuance of the experiment involved 114 of 300 mice, or 38 per cent. The death rate for each 5 day period is shown in Text-fig. 4.

Fourth to Tenth Replacements. Experiment 5.—From this point on small additions of new mice were made to the cages and the effects noted. They will not all be recorded in detail although the general effect of the new additions was deaths first among the new and then among the old. Table IV gives the size of each accretion and the mortality attached to each replacement group.

The death rate among the new mice was always in excess of that among the old. In short periods this is to be expected from the general rule that the new mice succumb first. In the table, mice surviving an exposure of 6 to 15 days are regarded thenceforth as old mice, whereas strictly speaking they are neither old nor new. Our impression is that mice exposed to an epidemic over 30 days are to be regarded as old.

TABLE IV.

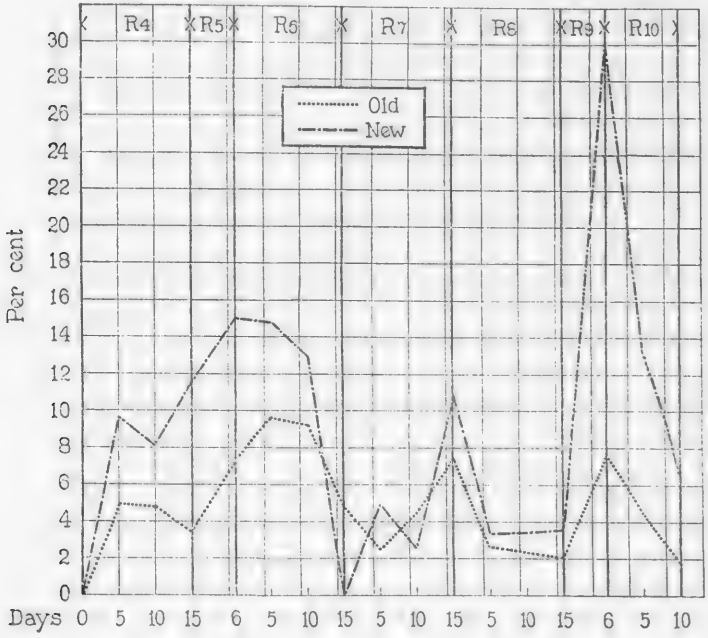
Deaths among Old and New Mice during the Fourth to Tenth Replacement Periods.

Period.	Duration of period.	Total No. of mice at beginning of period.	Old mice.	New mice.	Deaths during period.			
					Old mice.	Per cent.	New mice.	Per cent.
	days							
4	15	239	198	41	25	13	7	17
5	6	247	207	40	15	7	6	15
6	15	253	226	27	49	18	7	26
7	15	237	197	40	28	14	7	17
8	15	244	214	30	17	8	3	10
9	6	244	224	20	17	8	6	30
10	10	257	227	30	14	6	2	6

The general effect of the addition of the new mice is to maintain the death rate, as Topley has shown. The wave-like character of the fluctuations is evident from Text-fig. 5. The highest rates among the new mice are recorded in the sixth and ninth replacement periods; the interval between the beginning of the frequent replacement series and the sixth is 36 days, and between the sixth and the ninth is likewise 36 days.

Eleventh Replacement, or R 11. Experiment 6.—The effect of the addition of 30 new mice to the 227 old mice of the tenth replacement was negligible, as during 10 days only 1 mouse died; however, within the same time 13 of the old mice succumbed to mouse typhoid. At this point, July 15, 1921, what will be called the eleventh replacement was begun. At the time there were 62 cages in the mouse

village containing 183 mice. Each cage was recruited up to its normal content of 5 animals, thus giving a total population of 310 mice, of which 183 were old and 127 new. The death rate rose sharply among the new mice, and this was succeeded, after an interval of 5 days, by a rise among the old. The higher death rate among the new mice ceased at the 25th day, but later continued at about the same low level as that among the old during the succeeding 50 days (Text-fig. 6). The total mortality from mouse typhoid was 30 per cent, or among the new 31



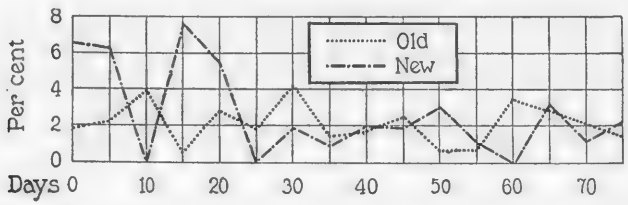
TEXT-FIG. 5. Death rate by 5 day periods among the old and new mice during the fourth to tenth replacement periods, inclusive.

per cent and the old 29 per cent, or as expressed in a proportion of old and new mice succumbing 52:40.

Twelfth Replacement, or R 12. Experiment 7.—October 18, 1921. The twelfth or final replacement was made in such a manner as to give the bacilli ample opportunity to infect new normal mice by retaining a small number of old mice and supplying five times as many new. All of the old mice in Series L and M, and all but 61 of Series K were discarded. To the latter there were added 54 new normal mice, bringing the total of the K series to 115. Series L and M were entirely

replaced by new mice, 100 in each. The three new series, K, L, and M, were now brought together in their usual order. The total number of mice was now 315, of which 254 were new and 61 old remaining from Series K. The ratio of old to new was about 1:4. The series was kept under observation for 60 days with the following results.

2 new mice were killed in fighting on the 1st day, reducing Series K to 113. The total number dying of mouse typhoid infection was 18. Mouse Typhoid II (the epidemic strain) was isolated from 12, and Mouse Typhoid I was isolated from 6. Of the latter, 3 were replacement or new mice.



TEXT-FIG. 6. Death rate by 5 day periods among the old and new mice during the eleventh replacement period.

TABLE V.

Deaths among Old and New Mice during the Eleventh and Twelfth Replacement Periods.

Period.	Duration of period.	Total No. of mice at beginning of period.	Old mice.	New mice.	Deaths during period.			
					Old mice.	Per cent.	New mice.	Per cent.
	days							
11	75	310	183	127	52	29	40	31
12	60	313	61	252	12	20	24	9

The total mortality due to Mouse Typhoid II was therefore 12 out of 113, or 11 per cent, as follows: 9 deaths, or 15 per cent, among the 61 old mice, and 3, or 6 per cent, among the 52 new mice.

Among the 200 mice, all new, in Series L and M, the mortality was 10 and 7 per cent respectively. Mouse Typhoid II was isolated in each instance. The replacement and death figures of Periods 11 and 12 are shown in Table V.

The failure of the addition of new mice to bring about the usual response is doubtless to be explained by the fact that the bacilli were of low virulence.

The deaths due to Mouse Typhoid I call for some explanation. In the preceding series this strain was occasionally isolated, so that it was known to us that it had come in, probably, through stock mice. It is to be supposed that among the 61 old mice taken from Series K there were some carriers of Mouse Typhoid I, and that it was transferred to the new mice added. However, the infection did not spread to the new mice in Series L and M, as Mouse Typhoid II was isolated from each mouse dying in these two series.

The cultures of the spleens from 30 of the mice dying in the twelfth replacement were plated, and from each plate from ten to fifteen colonies were picked. Of the 400 cultures thus obtained, all belonged to Mouse Typhoid II as shown by agglutination with monovalent antiserum.

Mouse Typhoid Carriers.

On September 23, 1921, the total number of surviving mice of Series K, L, and M of the eleventh replacement period was 208, distributed as follows: Series K 79; Series L 70; Series M 69. To them calomel and castor oil were administered, mixed with food so that each mouse received approximately 4 mg. of calomel and 1 cc. of castor oil. The next day portions of the loose stools were easily collected on a platinum loop and cultured. Fourteen positive cultures of bacilli belonging to the Salmonella group were obtained. Of the colonies subcultured and tested for agglutination, only one reacted positively with the antiserum of Mouse Typhoid II. The remaining thirteen were not identified immunologically. This result is reported for two reasons, first, as again indicating the occurrence of members of the Salmonella group in the intestinal contents of so called normal mice, and second, that the detection of carriers of any particular Salmonella organism used for experiment cannot be based on cultural properties alone. Since only five colonies were picked from each plate, it is possible, of course, that the subculturing of many colonies from the stools of the mice found to be positive for *Bacillus typhi murium* might have yielded organisms agreeing immunologically with the strain employed in the experiments.

Immunity.

In a study conducted concurrently with certain of the observations presented in this paper Webster[10] found that mice which had been fed sublethal doses of cultures of *Bacillus typhi murium* were rendered resistant or immune to doses *per os* of the living culture fatal to control mice. This state of increased resistance depended not on local conditions in the intestine but on a general state of immunization attended by the appearance of agglutinins in the blood. It is well known that mice may be protected, at least partially, from parenteral inoculations of the bacillus of mouse typhoid by the injection of killed cultures, and in view of the general immunization thus induced and arising from the ingestion of the bacilli under conditions not leading to death from mouse typhoid, it was deemed desirable to test the survivors of certain of the replacement series with lethal doses of *Bacillus typhi murium* injected intraperitoneally.

Experiment 8.—September 29, 1921. The survivors of the various replacement series L and M, including all periods except R 3, were chosen for the purpose. They numbered in all 128 mice which had been exposed to mouse typhoid from 2 to 18 months before and continued to live in the presumably contaminated surroundings. As controls for this series were taken 10 mice which had been fed 4 months previously on pure-line strains of *B. typhi murium* II without showing any effects or suffering any fatalities, and 40 contact mice in the same series (Q) in which no deaths occurred.[2]

Each mouse received an intraperitoneal injection of 1 cc. of salt solution containing 1:10,000 of an 18 hour agar slant culture of Mouse Typhoid II. This strain had been injected intraperitoneally into mice and recovered twenty-four times, and in doses of 1:30,000 to 1:10,000 regularly caused death of all mice within 200 hours.

All mice in the test except the 10 fed mice were obviously ill within a short time after injection. The results are shown in Table VI.

The results were decisive and indicate that the surviving exposed mice either have not acquired increased resistance to *Bacillus typhi murium* or do not show it by this method of testing. Judging from the manner of spread of mouse typhoid in the replacement experiments from the new to the old mice, of which some had passed through several successive outbreaks, our inference is that

[10] Webster, L. T., *J. Exp. Med.*, 1922, xxxvi, 71.

no definite protection[11] is accomplished by long survival in epidemics. However, protection was conferred on the 10 mice which had received large numbers of living bacilli with milk.

TABLE VI.

Results of Intraperitoneal Injection of 1:10,000 of an 18 Hour Agar Slant Culture of Mouse Typhoid into Mice Surviving Epidemic Periods R to R 11.

Mice.	No. injected.	No. dead at 18th day.	Per cent dead.
Survivors of epidemic periods R to R 11	128	108	84
Controls.....	40	30	75
Mice previously given large doses of viable single cell strain A by mouth	10	0	0

DISCUSSION.

The results presented in this paper as well as in the first article[2] of this series can be reproduced regularly under the conditions of the experiments, and thus constitute a succession of events to which the term rule may be applied.

It is perhaps desirable to restate in brief just what the events are which have in common the feature of the exposure of successive batches of mice to a disease, mouse typhoid, initiated purposely in a small number of animals through the ingestion of living mouse typhoid bacilli.

In three separate and distinct experimental attempts to induce outbreaks of mouse typhoid resembling the epidemic spreads sometimes witnessed in breeding stocks and thus spoken of as arising spontaneously, the course of the disease produced was of the nature of a sporadic prevalence. That this preliminary outbreak of disease was of this kind is shown not only by the number of mice dying of it but also by the number of cages attacked.

Once, however, this state of sporadic occurrence of mouse typhoid is inaugurated among a mouse population, all that appears necessary in order to convert the occasional deaths with low cage attack rate

[11] Agglutination tests were not made. It is probable that some of the mice possessed blood agglutinins, as it is recalled that in 66 per cent of the tested survivors of the first replacement, the serum agglutinated the epidemic strain.

into frequent deaths with high cage attack rate, is to bring into contiguous relation with the infected population increments of new and healthy mice not previously exposed to the disease.

The particular manner in which the sporadic instances of mouse typhoid arise indicates that very soon a wide distribution of the bacillary incitant takes place in spite of which the fatalities and the cage attack rate remain low.

The sporadic variety of prevalence is soon over. Just what might have taken place subsequently in these lots of mice, had nothing further been attempted, was not determined. No extended observations were made on the survivors, duly segregated, of these preliminary feeding experiments. It can, however, be surmised that under ordinary conditions additional mice would gradually have been added to the population through new births which, in turn, may have become infected, thus reinstating active infection.

But what is definite and significant is the fact that once the sporadic deaths have ceased, the bringing into this population of new lots of mice, which do not mingle directly with the old, suffices to provide the conditions favorable to a wide and even violent outbreak of mouse typhoid, as shown by the fatalities resulting.

The second epidemic spread, or new, in respect to its relative severity and in contrast to the sporadic occurrences, invariably progresses in a certain order. After an interval of about 5 days the new mice begin to succumb, the number of deaths and the proportion of cages attacked rising day by day. During the first period in which the new mice fall victims to the infection, the previously exposed or old mice do not show an increased death rate. But from the 10th to the 20th day following the addition of the new mice and hence the 5th to the 15th day after the new mice begin to succumb, the old mice are drawn into the wave of fatality with results varying somewhat in the different experiments but in which the old mice finally suffer a mortality to an equal, greater, or less degree.

The epidemic spread of the infection, as indicated by the deaths, tends to subside, and even to disappear entirely, before all, sometimes after only a small part, of the exposed mice have been destroyed. A tendency which is obvious in all the experiments is for the establishment of a state of equilibrium between the surviving mice and

the infecting bacillus. This equilibrium is overcome by the introduction of fresh infectible mouse material, resulting in an outbreak of mouse typhoid. The undulations, or epidemic waves, of the disease thus induced in the different replacements are remarkably uniform. The cessation of the undulation, as indicated by the deaths

TABLE VII.

Number of Mice from Preceding Periods Living at the Time of the Twelfth Replacement Period.

Replacement period.	No. of mice living.	Original No.	Deaths.
			per cent
R 1	23	178	87
R 2	3	228	99
R 3	1	114	99
R 4*	17	41	58
R 5	6	40	85
R 6	6	27	78
R 7	8	30	73
R 8	10	30	67
R 9	17	20	15
R 10	21	30	30
R 11	87	127	31

*Replacements 4 to 10 covered short periods of 6 to 15 days each. The total period covered by these seven replacements was 82 days.

is not abrupt but gradual, although the late fatalities may not be due to delayed infection so much as to protracted illness and long survival.

The experiments show definitely that mice which have weathered the storm of the epidemic in the sense of having passed, without succumbing, through one or more violent outbreaks of the disease, are not insured against eventual fatal attack. This fact is brought out in Table VII, which shows that as successive epidemic waves pass over the always changing population, the old or previously exposed mice tend ultimately to be wholly wiped out.

In due time the precise conditions concerned in the conversion of the incipient and sporadic into epidemic spreads of disease will need to be considered narrowly. We have learned in respect to the enteric infection mouse typhoid how the one may be turned into the other

and that the epidemic outbreaks are always, as it were, frustrated before all the available infectible material is consumed.

Obviously there are two outstanding factors which affect and determine the state of the microbic incitant in its relation to the host: the one relates to the quality or virulence, so called, the other to the quantity or number. Hitherto it has been the virulence factor that has been most considered and invoked to account for the wave-like movement of epidemic disease. This fact will appear prominently in the brief review of the literature which is to follow. The second or quantity factor has been much less discussed. As pointed out in the body of this paper, we believe that it cannot well be disregarded. Perhaps the disease mouse typhoid may provide favorable experimental materials for just this study. Our experiments show decisively that immediacy of contact is by no means a necessary condition for the wide diffusion of the microbic incitant of mouse typhoid among a mouse population maintained in small segregated groups; and this fact is emphasized also by the occurrences of the spontaneous epidemic described by Lynch.[1] The mere rapidity of the fluctuation of the curve of the death rate in the replacement experiments either indicates that the virulence factor is highly unstable, or that some other attending condition acts to modify or control the results as measured by infection and death.

Thus far attention has centered on the factors affecting the microbic incitant, while those which may be presented by variation in the host have yet to be considered. Difficult as may be the unravelling of the former, for which certain methods possibly are at hand, the other has been regarded next to insuperable. Aside from choosing a stock roughly homogeneous and covering the matter of size and age, nothing has been attempted as regards the host. In a recent study[12] of tuberculous infection in guinea pigs an effort has been made to obtain values for certain hereditary qualities, an aspect of the problem of infection in mouse typhoid capable of treatment.

The relation of the carrier state to the host awaits elucidation. That carriers arise among the exposed mice which do not succumb early is demonstrated. Experience indicates that the detection

[12] Wright, S., and Lewis P. A., *Am. Naturalist*, 1921, lv, 20.

of carriers is determinable not by cultivation tests alone but chiefly through the reactions of immunity. Without the latter, error readily creeps into the results because of the presence in the intestine of apparently normal mice, of members of the broad group of organisms embraced under the loose term of mouse typhoid bacilli.[10] Webster's experiments show that the purposive introduction of living or even killed mouse typhoid bacilli into the intestines of normal mice, under proper conditions, leads to an immunization sufficing to prevent infection from otherwise fatal infecting doses of cultures for such mice. This state of immunity is general in the sense that specific agglutinating bodies are demonstrable in the blood, and the immunized mice resist not only intrastomachic but also intraperitoneal injections of the living bacilli.

Review of the Literature.

The possibility of employing artificial epidemics among small rodents for the study of the spread of infection should date from the experiments of Danysz,[13] published in 1900. As will be recalled, he endeavored to exterminate rodents, rats especially, on a large scale through the employment of living cultures of a bacillus belonging to the so called mouse typhoid group. The method did not really succeed. The reasons for the failure are now apparent and, in the main, arise first from the rapid loss of infecting power or virulence of the cultures, and second from the circumstance that the bacillus as it occurs in the rat cadaver is less infective than at the moment infection takes place. Hence the devouring of the cadavers by their living companions arrests rather than propagates the disease. These unsuccessful results were foreshadowed by laboratory studies made by Danysz, who found that no matter how highly active the cultures were at the outset, their power of infection by feeding did not extend beyond a very few passages. Danysz' conclusions may be stated as follows:

Cultures may be enhanced so that they will induce fatal infection on ingestion, but the increased activity will not endure beyond the third or fourth passage; exposure of a group of non-infected mice to a small number of mice purposely fed on a virulent culture will induce fatal infection in most but not in all of the former. The few survivors succumb to a later direct feeding of culture of average virulence. Cultures enhanced by organ and blood passages suffer concomitant diminished activity when administered *per os*. Cultures obtained from the blood and organs in the incubation stage of infection are more virulent than when derived

---

[13] Danysz, J., *Ann. Inst. Pasteur*, 1900, xiv, 193.

from cadavers. Resistance or susceptibility to infection among small rodents is affected by race and age, and even by individual peculiarities.

Later, Bainbridge[14] noted that a spontaneous epidemic among wild rats kept under experimental conditions may lead to the death of all, and as the conditions approach those of nature, the death rate becomes less. The percentage of deaths noted by Bainbridge was not affected by the number of rats devoured, and he considered the rat to possess a preexisting immunity which arises through the occurrence of spontaneous epidemics among those animals. In support of this view, rats surviving the feeding of cultures were found to carry agglutinating properties in their blood for the bacilli used.

The conditions favoring the perpetuation of the pathogenic proclivities of the mouse typhoid bacilli have been investigated recently by Topley. He has utilized his material, as we had planned to use ours, in an endeavor to penetrate into the hidden recesses of epidemiology. He repeated Danysz' experiments, which he confirmed, but he went beyond the latter in acting on the supposition that the consumption of food soiled by excreta may be a more common and effective mode of infection than the devouring of cadavers carrying already depressed bacilli.

From the first, Topley[15] looked upon the spread of epidemics and their rise and fall as having to do primarily and essentially with the infecting power of the inciting microbe. The rapid fluctuations of the mouse typhoid bacillus in this regard seemed to provide the needed explanation of the failure to presage epidemics among mice. He sought to supply the needed conditions by starting an epidemic through feeding and then adding, in a large cage, daily small complements of normal mice, in order, through passage, to keep the bacilli alive and actively infective. In this way the tendency for the disease, once started, to cease abruptly was overcome, and the infection would proceed in regularly recurring waves, apparently without time limit. He further observed that the deaths occur in large groups with intervals between, during which the deaths are few or far between. He distinguished two phases according to which the new mice added tended to succumb or survive, and these phases are the rise of a wave when infection is likely to occur and its fall when it is likely to fail. Finally, he noted that mice which have passed successfully through one epidemic wave may succumb to a later one, and an infected population to which no additions are made will outlive one into which fresh mice are brought, while the ultimate survivors have not escaped infection and a considerable part has become carriers. A substitution of strains took place during Topley's experiments as in ours. He began with a strain of Gärtner's bacillus, but as his experiments progressed a second organism, identified as belonging to the suipestifer group, intervened. Subsequently strains

---

[14] Bainbridge, F. A., *J. Path. and Bact.*, 1909, xiii, 443.

[15] Topley, W. W. C., *J. Hyg.*, 1920-21, xix, 350.

of Gärtner's bacillus, or of this second organism, or both together, were obtained from the great majority of the dead animals examined.[16]

In a second communication[17] the outbreak of an epidemic among a normal stock, probably through accidental spread from cages with infected mice, is described. Not all the exposed mice succumbed and the deduction made was that the infection was not of "uniform intensity, passed from a healthy carrier to one or more susceptible individuals, but that some process is set in motion, which results in an increase in the infectivity of the parasite, this in its turn giving rise to a fresh wave of mortality among the cage population."

Topley's experiments were arranged to achieve the greatest likelihood of infection occurring by providing the greatest possible concentration of contaminated materials; namely, dejecta carrying the bacilli or cadavers devoured by their companions. Our experiments were long under way before Topley's main publications appeared in 1921. We had in mind not the providing of optimal conditions for infection to take place in mice, but the imitation, if only roughly, of those occurring naturally in man and in laboratory animals, in connection with which epidemics of disease occur.

SUMMARY.

1. A kind of mouse village was set up into which was introduced a small number of mice fed on a culture of so called mouse typhoid (*Bacillus pestis caviæ* of the *Bacillus paratyphosus* B group) bacillus. The spread of the infection so induced to the cages, or "homes," of the other mice was left to accident through the attendant who fed the animals and cleaned the cages. That this means was likely to be sufficient was deduced from the epidemic reported by Lynch.[1] A spot map was kept throughout the experiments which extended from 1919 into 1922.

2. The first effect of the exposure of normal mice to a much smaller number of mice fed on the culture is to set up a *sporadic*,

[16] Topley and his coworkers (Topley, W. W. C., Weir, H. B., and Wilson, G. S., *J. Hyg.*, 1921, xx, 227) have studied these two organisms serologically, along with other related bacilli, and suggest that the relations between the Gärtner's bacillus and *B. suipestifer* group of organisms is so close that for purposes of classification and nomenclature the name of *B. enteritidis* may, for convenience, be applied to the whole group.

[17] Topley, W. W. C., *J. Hyg.*, 1921, xx, 103.

not an *epidemic* outbreak of mouse typhoid. This is the regularly recurring incident of the experiment as shown by low mortality and low cage attack rate. Such a sporadic prevalence is self-limited in time.

3. The introduction of fresh, normal mice into a community in which sporadic deaths are occurring leads regularly, not to the further extension of the sporadic deaths, but to an epidemic spread, as shown by high mortality and high cage attack rate. The epidemic begins with deaths among the new mice, but extends to the old mice which succumb later. The spread ceases and the wave subsides before all the mice have succumbed. A state of equilibrium between the infecting bacillus and the surviving mice is reached; no more deaths occur. The epidemic outbreak, therefore, is also self-limited in time.

4. If, now, another new addition of normal mice is brought into the potentially infected community, the events are reenacted; deaths occur among the new, another epidemic wave sweeps through the population, again claiming victims among the previous survivors.[18] Through the replacement of the destroyed mice with fresh, normal mice, epidemic wave after wave is produced, until certain groups of old survivors are entirely wiped out. There seems to be no limit to this process, as there will always be survivors at least of the later groups added.

5. The dying down of the epidemics and the attaining of the equilibrium do not mean the elimination of all the bacilli. Potential infection still lurks in mouse "carriers" and on the hands of the attendant. No successful method of completely removing the bacilli from the hands of the attendant was found.

6. The maintaining of the epidemic waves is dependent on the presence of new lots of mice, whether supplied from without or produced within through new births. It is the latter sources which provide the consumable material in such natural epidemics as that described by Lynch.[1] Hence their slower movements as compared with the epidemic spreads and rise and fall of the epidemic waves in the artificially propagated instances in which new batches of mice are brought into the village in bimonthly intervals.

[18] In the third replacement period a rise in death rate constituting a small epidemic occurred after the 60th day and independent of the addition of new mice. No explanation has been found for this occurrence.

7. The evidence at hand is to the effect that the degree of infectivity of "mouse typhoid" bacilli is highly fluctuating, and it appears that all the bacilli which are included under that name, classed variously as *Bacillus enteritidis*, Gärtner's bacillus, *Bacillus paratyphosus* B, *Bacillus suipestifer*, and *Bacillus pestis caviæ*, infect mice in a similar, possibly indistinguishable manner, inducing self-limited outbreaks of disease reaching at times epidemic proportions. This quality of infectivity, or virulence, is one factor in the process but alone does not suffice to account for the observed facts. A second influence is not improbably quantity, or dosage, of the inciting microorganism. Attention to the manner of spread of an epidemc, as revealed graphically by the spot map, shows that it is never uniform but patchy. The supposition is, therefore, that among the new mice are certain individuals so highly susceptible as to react to small numbers of bacilli of average infectivity. Within these animals the multiplication is rapid, so that a wider spread of much larger amounts of these average, or even temporarily enhanced bacilli takes place with the inevitable effect of communicating, through greater dosage, etc., the infection to other and less susceptible individuals among the new and also the older lots. This process continues so long as rapid multiplication can occur. This latter is, in turn, determined by the innate tendency of the bacilli to return to an average of infectivity and by mouse individuals of greater resistance to restrict free growth and multiplication. In this manner, possibly, the epidemic spread is checked and the curve representing it made to fall more or less quickly to a base-line.

EXPERIMENTS ON NORMAL AND IMMUNE MICE WITH A BACILLUS OF MOUSE TYPHOID.

By LESLIE T. WEBSTER, M.D.

(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

(Received for publication, December 7, 1921.)

As part of a wider study of the phenomena of epidemics among animals described by Flexner[1] and Amoss,[2] the following series of experiments was undertaken to ascertain the varieties and degrees of resistance in normal and immunized mice to mouse typhoid bacilli of the paratyphoid-enteritidis group. The experiments were so designed as to bear directly on such questions as the relation of infectivity on the part of the microorganisms to the portal of entry into the body of the host and also on the influence of a possible local as opposed to a general immune state, the effect of which would be to alter or even to abate the danger of infection by the ordinary route traversed in nature.

Since the problem of infection, as manifested in the higher animal species, is a complex made up of potentialities in the host and in the microbe, it becomes necessary to control and keep constant as many of the factors as is possible. Hence the mice, chosen from healthy stock with practically no death rate, were of one age, often from the same litter, of practically the same weight, and were kept under close observation before and during experiments. The stock room diet of bread and milk was continued throughout the experiments. The mouse typhoid strain, M. T. II, obtained from an experimental epizootic studied by Amoss,[2] was identified with *Bacillus pestis caviæ* Smith and closely related to *Bacillus aertrycke* (mutton) of Schütze.[3] This organism was carried in stock and maintained by monthly stab cultures in tube agar of pH 7.4. In order to secure a culture of

[1] Flexner, S., *J. Exp. Med.*, 1922, xxxvi, 9.

[2] Amoss, H. L., *J. Exp. Med.*, 1922, xxxvi, 25, 45.

[3] Webster, L. T., *J. Exp. Med.*, 1922, xxxvi, 97.

Strain M. T. II of a degree of virulence adequate to infect regularly *per os*, it was necessary to pass it through a series of mice by intrastomachal inoculation. At autopsy, to guard against substitution, the identity of the recovered bacilli was always established by means of fermentation and agglutination tests. Dosage was determined by making use of the fact that cultures of Strain M. T. II in plain broth of pH 7.4, when inoculated in small amounts, reached, after 14 to 24 hours incubation at 37°C., a relatively constant number; *e.g.*, 1 billion per cc. With a 14 to 24 hour broth culture the approximate number of organisms contained in any dilution could thus be calculated.

Effects on Normal Mice of Intrapleural, Intraperitoneal, and Intrastomachal Inoculations.

Intrapleural Inoculation with Culture M. T. II.—These experiments and others to follow were planned in order to ascertain the manner of resistance of mice to intrapleural injections of the cultures. The tests were made as follows:

9 mice with controls were given intrapleurally 0.2 cc. of a 16 hour broth culture of Strain M. T. II obtained from the stock agar tube. Interpreting these and all subsequent dilutions on the 1 cc. basis, 3 mice received 1:5,000 dilution, 3 received 1:50,000 dilution, and 3 received 1:500,000 dilution. At 2 hour intervals small amounts of fluid were aspirated from the infected pleural cavity. Films were made and 1 drop was transferred into fluid agar (48°C.), shaken, and poured. Colonies were counted after 24 hours incubation (37°C.). Table I summarizes the duration of life of experimental animals; the controls ran a similar course.

TABLE I.

Duration of Life of Mice Injected Intrapleurally with Strain M. T. II

Dilution.	Duration of life in Experiment 2.	Duration of life in Experiment 3.	Duration of life in Experiment 4.	Average duration of life.
	days	*days*	*days*	*days*
1:5,000	5–1–3	1–6–3	1–1–3	2+
1:50,000	5–5–6	Survived*–3–5	4–10–7	5+
1:500,000	6–7–5	15–9–14	10–6–6	8+

* Survivors were discarded after 30 days.

The counts of the colonies on the plates, although not quantitatively accurate, were, nevertheless, of value in showing the relative changes in numbers. Such sources of error as dry puncture, irregularity of distribution of bacilli in the pleural exudate, and variation in size of drop, did not obscure the tendency of the bacteria after an initial lag of 4 to 6 hours to increase rapidly in number until the mice were overwhelmed (Table II and Text-fig. 1).

TABLE II.

Bacterial Growth in Vivo (Intrapleural).

Dilution 1:50,000; number of colonies in plate 150 to 200.

Length of time after injection.	Mouse 1.				Mouse 2.				Mouse 3.			
	Condition.	Smear.		No. of colonies in plates.	Condition.	Smear.		No. of colonies in plates.	Condition.	Smear.		No. of colonies in plates.
		Cells.	Phagocytosis.			Cells.	Phagocytosis.			Cells.	Phagocytosis.	
hrs.												
1	Well.	±	0	100	Well.	±	0	30	Well.	±	0	60
2	"	±	0	100	"	±	0	50	"	±	0	100
3	"	±	±	50	"	0	0	50	"	0	0	40
4	"	±	+	100	"	0	0	100	"	0	0	100
5	Sick.	+	+	150	Sick.	+	+	250	Sick.	0	0	15*
6	"	+	?	250	"	+	+	250	"	?	?	250
24	"	++	+	750	"	+	+	250	"	+	?	150
48	"	++	+	750	"	+	?	250	"	?	?	250
72	"	++	+	1,200	"	++	+	720	"	+	+	450
98	"	?	?	1,620	"	+	?	690	"	+	+	2,000
119	Dead.				Dead.							
143									Dead.			

* Probably an error.

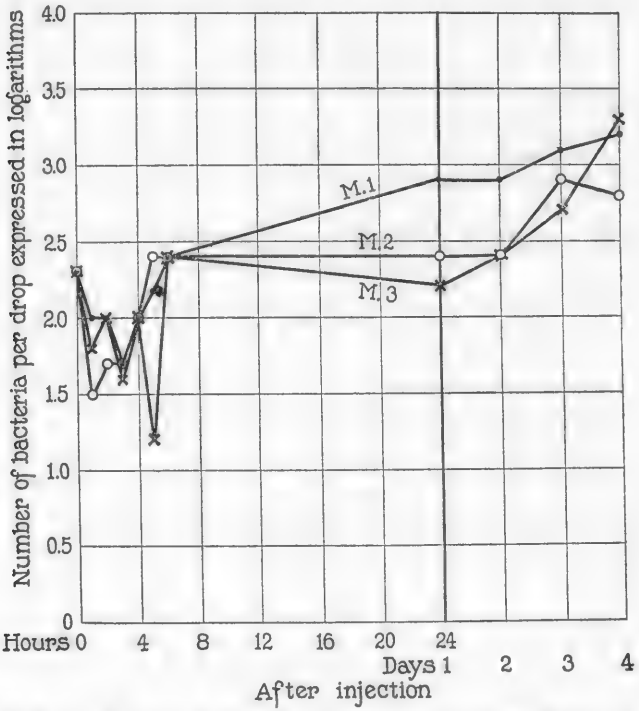
The series of events following the intrapleural injection of fixed doses of a constant culture was quite regular, as was shown by repetition of the experiment just described.

Intraperitoneal Inoculation with Culture M. T. II.—This experiment was the counterpart of the pleural one immediately preceding. It was carried out as follows:

6 mice with controls were injected intraperitoneally with 0.2 cc. of a 16 hour broth culture of Strain M. T. II obtained from the stock agar tube, 3 receiving 1: 5,000 and 3, 1:50,000 dilution. At short intervals small amounts of fluid were

aspirated from the peritoneal cavity. Films were made and 1 drop of the exudate was plated in the fluid agar (48°C.) medium. The colonies were counted after 24 hours incubation (37°C.).

The result is similar (Table III); after an initial lag, the bacilli multiply rapidly until they reach a high number at about the time death occurs, as is shown in Text-fig. 2.



TEXT-FIG. 1. Bacterial growth *in vivo* (intrapleural). The figures are taken from Table II.

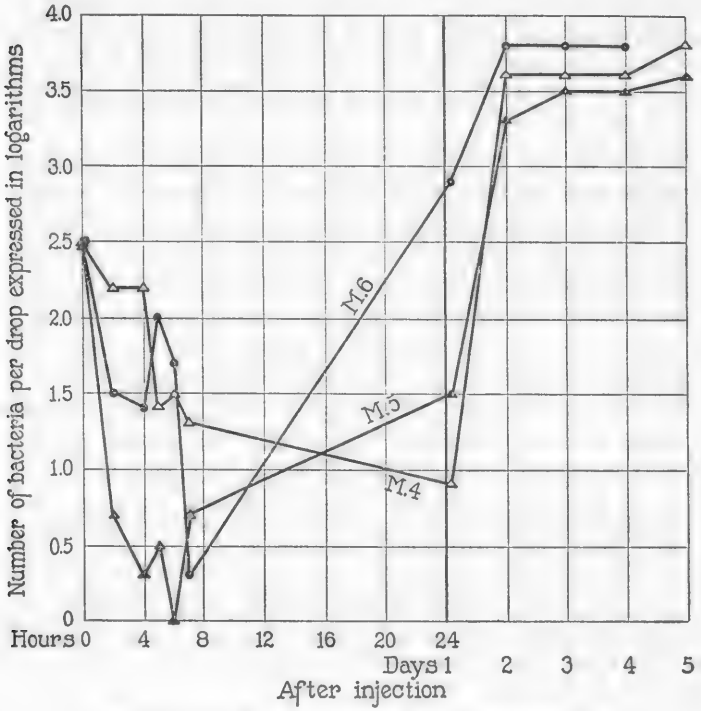
The events in the two experiments with pleural and peritoneal inoculation can be summarized as follows: if live cultures of this organism are injected intrapleurally or intraperitoneally into normal mice, there occurs an initial lag in the rate of bacterial multiplication lasting 4 to 6 hours, followed by a rapid and continual acceleration of

TABLE III.

Duration of Life of Mice Injected Intraperitoneally with Strain M. T. II.

Dilution.	Duration of life in Experiment 9.	Duration of life in Experiment 10.	Average duration of life.
	days	*days*	*days*
1:5,000	6–3–5	10–10–Survived.*	7
1:50,000	7–6–5	13–5–13	8+

* Survivors were discarded after 20 days.



TEXT-FIG. 2. Bacterial growth *in vivo* (intraperitoneal).

growth until the death of the animal. To this rule there are exceptions in an occasional recovery or an undue prolongation of the survival period. The mice dying acutely exhibit few pathological changes,

while those in which the infection pursues a more chronic course show macroscopically thick peritoneal or pleural exudation, and lesions of the lungs, liver, and spleen consisting of hepatization, nodule formation, swelling, etc. The injected bacillus was always recovered from the heart's blood and organs.

Intrastomachal Inoculation with Culture M. T. II.—A fixed dose which ranged from 0.5 to 1 cc. of the broth culture diluted 1:100 was injected into the stomach through a stiff silver catheter attached to a suitable measuring syringe with rubber tubing. This slight procedure which assures ingestion of the culture can be carried through expeditiously and securely. Culture M. T. II could be kept at an approximately constant infecting level if passed through mice *per os* at frequent intervals. The injections *per os* were made about 3 hours after feeding.

Thus far, in spite of the control exercised over the origin and selection of the mice and the uniform treatment and dosage of the culture, some degree of fluctuation has attended the experiments in infection of normal mice *per os*. That irregularities would enter into this part of our studies was of course foreseen. It is highly probable that under ordinary conditions of propagation of an epizootic among mice by a representative of the paratyphoid-enteritidis group, infection takes place always *per os*, and yet we know that survivors invariably occur. Our experiments show that the survivors have no necessary relation to dosage of a given constant culture, although on the whole the animals receiving the larger doses are the ones tending to succumb. Table IV is intended to bring out the variations met with in a number of carefully planned tests.

In addition to the irregularities shown in these experiments, it happens that still greater ones are met with when instead of introducing the culture directly into the stomach of mice, the organisms are added to the food by soaking up the broth culture with bread which is then fed to the animals.

For this test the mice were assembled 6 per cage, and it was noted that they devoured the soaked bread voraciously on successive days without necessarily succumbing to infection. Table V summarizes the results of two such feeding experiments.

TABLE IV.

Variation in Susceptibility Following Injection per Os (Stomach Tube).

No. of mice.	Broth dilution.	Duration of life in fatal cases.	No. of survivors.	Result.
		days		
1	1:100	9	0	
2	1:200	12–16	0	Regular.
1	1:500		1 (60 days).	
3	1:100	8–14–20	0	
1	1:1,000		1 (60 days).	Regular.
1	1:10,000		1 (60 ").	
3	1:100	19–21	1 (60 ").	
1	1:1,000		1 (60 ").	Regular.
1	1:10,000		1 (60 ").	
3	1:50	12–43	1 (60 ").	
3	1:500	11	2 (60 ").	Irregular.
3	1:5,000	17–18–23	0	
3	1:50,000		3 (60 days).	
2	1:1	18	1 (30 ").	
3	1:10	8–11–21	0	Irregular.
3	1:100	7–8	1 (30 days).	
2	1:1	4–10	0	
2	1:10		2 (18 days).	Irregular.
2	1:100	17–18	0	

TABLE V.

Variation in Susceptibility Following Injection per Os of Strain M. T. II (with Feedings).

No. of mice.	Duration of feeding.	Broth dilution.	Duration of life in fatal cases.	No. of survivors.	Result.
	days		*days*		
3	3	1:10,000	12	2 (30 days).	
3	5	1:10,000		3 (30 ").	Irregular.
3	7	1:10,000	17	2 (30 ").	
5	5	1:10,000		5 (40 ").	
5	7	1:10,000	11–11–17	2 (40 ").	Irregular.
5	9	1:10,000	15	4 (40 ").	
5	13	1:10,000	22–38	3 (40 ").	

Although the stomachal injections and the feedings of broth cultures of Strain M. T. II did not consistently induce infection but rather brought out factors of variation, themselves illuminating when considered in relation to observed epizootics arising accidentally among mice, yet certain general features may be gleaned from the tests performed. An incubation period of about 5 days preceding the indications of infection or death was noted. There was no invariable relation between dosage and fatal infection; without reference to dosage or culture certain mice proved refractory. The type of infection was predominatingly chronic with regular involvement of the intestine, spleen, and liver, and occasional involvement of the lungs and heart. In all instances Strain M. T. II was recovered from the heart's blood and organs.

Effects on Immune Mice of Intrapleural, Intraperitoneal, Subcutaneous, and Intrastomachal Inoculations.

The next tests related to the influence exerted by immunizing injections of dead or living bacilli on the subsequent inoculation of given doses of active cultures of Strain M. T. II. The experiments were arranged to bring out the effect not only of the immunizing action in general immunity but also of a possibly greater state of local immunity at the site of the protective inoculations. The live organisms were introduced into the pleural and peritoneal cavities and also into the stomach.

The killed cultures employed for the protective injections consisted of a 24 hour broth culture which had been heated to 55°C. for 2 hours and afterwards standardized and preserved with 0.3 per cent tricresol.

Intrapleural and Intraperitoneal Inoculation.—The killed bacilli, in doses of 250,000, were injected either into the right pleural or the peritoneal cavity, after which, at the expiration of a given period of time, living bacilli were injected into one or the other of these cavities, as shown in the protocols which follow.

12 mice were inoculated with 250,000 killed Strain M. T. II bacilli, 6 intrapleurally and 6 intraperitoneally, and 7 days later the inoculations were repeated. 9 days after the second inoculation the mice were divided into batches of 3 and injected with 0.2 cc. of a 1:1,000, 14 hour broth culture of Strain M. T. II either intrapleurally or intraperitoneally. 3 pleural "vaccinated" mice, along with 3

normal controls, were injected intrapleurally, another 3 of the pleural "vaccinated" mice, together with 3 controls, received the material intraperitoneally. At intervals, fluid was aspirated from the cavity into which the living bacilli were injected, films were made, and 1 drop was plated in agar. The colonies developing in the latter were counted after 24 hours growth. Tables VI to VIII inclusive and Text-fig. 3 give in brief the results of the experiment.

TABLE VI.

Duration of Life of Normal and "Vaccinated" Mice Injected Intrapleurally and Intraperitoneally.

Mice.	Mice "vaccinated" intrapleurally; injected intrapleurally.		Mice "vaccinated" intrapleurally; injected intraperitoneally.	
	Duration of life.	Average duration of life.	Duration of life.	Average duration of life.
	days	*days*	*days*	*days*
"Vaccinated".....	13–14–28	18+	14–17–25	18+
Controls.....	2–4–4	3+	1–1–5	2+

TABLE VII.

Bacterial Growth in the Pleural Cavities of Normal and "Vaccinated" Mice. Mice "Vaccinated" Intrapleurally; Injected Intrapleurally.

Length of time after injection.	14 hr. broth culture of Strain M. T. II; dilution 1:5,000; 5,000 organisms per drop.					
	Mouse 1, "vaccinated." No. of colonies in plates.	Mouse 2, "vaccinated." No. of colonies in plates.	Mouse 3, "vaccinated." No. of colonies in plates.	Mouse 4, control. No. of colonies in plates.	Mouse 5, control. No. of colonies in plates.	Mouse 6, control. No. of colonies in plates.
hrs.						
2	2	0	1	100	420	120
3	0	0	0	300	150	300
5	0	0	5	450	600	60
6	3	0	3	450	180	800
8	0	0	1	450	3,600	720
9	0	0	0	4,000	5,400	3,000
30	120	0	30	2,000	900	800
days						
2	0	0	10	5,000 Dead.	100	1,800
3	0	3	2		360	Dry tap.
4	180	40	10		Dead.	Dead.
5	240	25	150			
11	1,620	1,080	1,350			
13	Dead.					
14		Dead.				
23			1,440			
28			Dead.			

TABLE VIII.

Bacterial Growth in the Peritoneal Cavities of Normal and "Vaccinated" Mice. Mice "Vaccinated" Intrapleurally; Injected Intraperitoneally.

Length of time after injection.	14 hr. broth culture of Strain M. T. II; dilution 1:5,000; 5,000 organisms per drop.					
	Mouse 7, "vaccinated." No. of colonies in plates.	Mouse 8, "vaccinated." No. of colonies in plates.	Mouse 9, "vaccinated." No. of colonies in plates.	Mouse 10, control. No. of colonies in plates.	Mouse 11, control. No. of colonies in plates.	Mouse 12, control. No. of colonies in plates.
hrs.						
2	90	1	10	360	3,600	80
3	0	0	15	3	1,350	550
5	4	0	10	40	720	15
6	1	0	1	120	1,080	1,350
8	0	0	0	3,150	4,000	6,000
9	0	2	0	4,000	6,000	6,000
30	15	0	30	4,000	Dead.	Dead.
days						
2	20	0	Dry tap.	4,000		
3	0	15	" "	6,000		
4	150	360	" "	6,000		
5	180	90	" "	Dead.		
11	6	3,000	" "			
14		Dead.				
17	Dead.					
23			15			
25			Dead.			

TABLE IX.

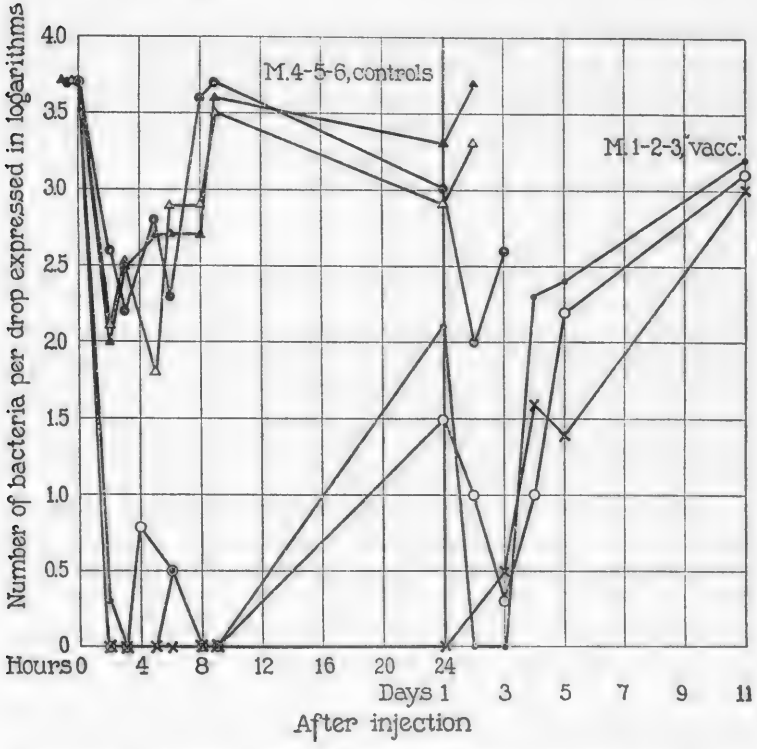
Duration of Life of Normal and "Vaccinated" Mice Injected Intrapleurally and Intraperitoneally.

Mice.	Mice "vaccinated" intraperitoneally; injected intrapleurally.		Mice "vaccinated" intraperitoneally; injected intraperitoneally.	
	Duration of life.	Average duration of life.	Duration of life.	Average duration of life.
	days	*days*	*days*	*days*
"Vaccinated."	15–17–19	17	21–36–Survived.*	32+
Control.	4–4–1	3	3–4–4	3+

* Survivors were discarded after 40 days.

This experiment was paralleled by a similar one in which the "vaccination" had been made intraperitoneally, followed as before by the injection of living bacilli into the pleural or peritoneal cavity. The results in general were identical

with the preceding: Table IX shows the duration of life in the series and the curves illustrating bacterial growth in "vaccinated" mice and controls are similar to those of Text-fig. 3.



TEXT-FIG. 3. Bacterial growth in the pleural cavities of normal and "vaccinated" mice.

The experiments may be summarized as follows: Living bacilli injected into the control mice showed the usual lag of 4 to 6 hours, followed by a rapid increase in number until the death of the animal. These mice succumbed within 3 days after injection, showed few lesions at autopsy, and positive cultures from the heart's blood and organs. Living bacilli injected into the "vaccinated" mice were partially destroyed and held in check by the protective mechanism of

the animal body for 2 or 3 days. Subsequently the rate of bacterial multiplication increased gradually until the death of the animal. These mice succumbed within 18 days after injection, and showed at autopsy extensive local lesions as well as marked involvement of the spleen and liver. Cultures from the heart's blood, exudates, and organs were positive. The partial immunity following this type of treatment is entirely of a general nature; no essential difference in the progress of infection or ultimate protection is to be noted whether the mice are given the injection into the cavity which previously received the "vaccine" or into the cavity previously "unvaccinated."

Subcutaneous Inoculation.—

As a corollary to the above, 6 mice were "vaccinated" subcutaneously with a total of 150,000,000 bacilli given in two doses. 5 days after the second inoculation, twice the usual dose of living bacilli was given to 3 of the mice intrapleurally, 3 intraperitoneally, and to normal controls. Because of the larger dose of living bacilli, the controls died within 24 hours, while the average survival period of the "vaccinated" mice was 17 days. The progress of the infection, however, was to all intents and purposes identical with that in the mice "vaccinated" by way of the pleura and peritoneum and their controls.

Inoculation per Os.—The ordinary, or as we say, natural mode of infection with mouse typhoid bacilli is by way of the gastrointestinal tract and hence the influence of the "vaccination" on infection *per os* was next studied. Since the preceding tests all indicate that the effects of protective inoculation with killed cultures on the progress of a subsequent injection of living bacilli are practically identical, however the "vaccination" is carried out, namely by the pleural, peritoneal, or subcutaneous route, the inoculation of dead bacilli in this experiment was made subcutaneously.

150 normal mice of approximately the same weight (16 to 18 gm.) and age (2 months) were segregated. To 120 of them 0.5 cc. of a 24 hour broth suspension of Bacillus M. T. II, previously heated to 55°C. for 2 hours, was given under the skin. The "vaccine," preserved with 0.3 per cent tricresol, was standardized to contain 500,000,000 bacilli per cc., so that each mouse received an initial dose of approximately 250,000,000 dead bacilli. 30 mice were set aside to be used as controls.

After an interval of 13 days, 10 of the mice having received one injection of the "vaccine" were given an intraperitoneal injection of 0.5 cc. of a living 14 hour broth culture of Mouse Typhoid II, diluted 1:100, and a second 10 of the "vaccin-

ated" mice received the same dose into the stomach by silver tube. Controls were injected intraperitoneally and *per os* with doses of 0.5 cc. of the same broth culture diluted 1:50 to 1:250.

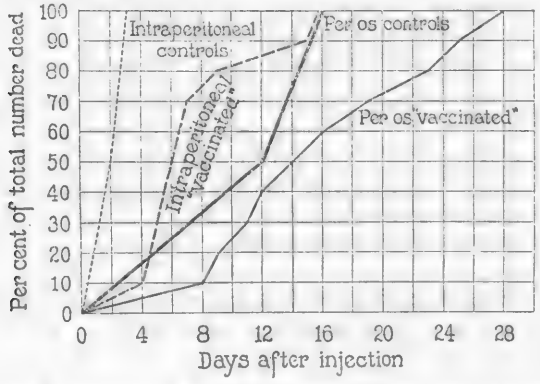
TABLE X.

Duration of Life of Normal and "Vaccinated" Mice Injected Intraperitoneally and Intrastomachally.

Mice.	No. of mice.	Dosage.	Duration of life.	Average duration of life.
			days	*days*
Intraperitoneal control.	1	1:100	1	1
" "	2	1:200	2–3	2.5
" "	1	1:500	4	4
" "vaccinated."*	10	1:200	4–5–5–6–6–7–7–9–15–16	8
Per os control.	1	1:100	9	9
" " "	2	1:200	12–16	14
" " "	1	1:500	Survived.†	
" " "vaccinated."*	10	1:200	8–9–11–12–14–16–19–23–25–28	16.5

* The "vaccinated" mice had received one subcutaneous injection previous to the intraperitoneal or intrastomachal injection.

† Survivors were discarded after 62 days.



TEXT-FIG. 4. Duration of life of "vaccinated" mice injected intraperitoneally and intrastomachally. The figures are taken from Table X.

Table X and Text-fig. 4 summarize the results of this experiment, which can be expressed as follows: A single subcutaneous inoculation

of killed bacilli into mice confers a partial protection against subsequent introduction of live organisms *per os* as well as by way of the peritoneal cavity.

The remaining 100 "vaccinated" mice received a second subcutaneous injection of the killed cultures 14 days after the first. After the expiration of another 18 days, 20 of these mice were given 1 cc. of a 14 hour living broth culture of Strain M. T. II diluted 1:100 into the peritoneum, and 20 the same dose by stomach tube. Table XI and Text-fig. 5 summarize the results of the injections in these animals and in the controls.

TABLE XI.

Duration of Life of Normal and "Vaccinated" Mice Injected Intraperitoneally and Intrastomachally.

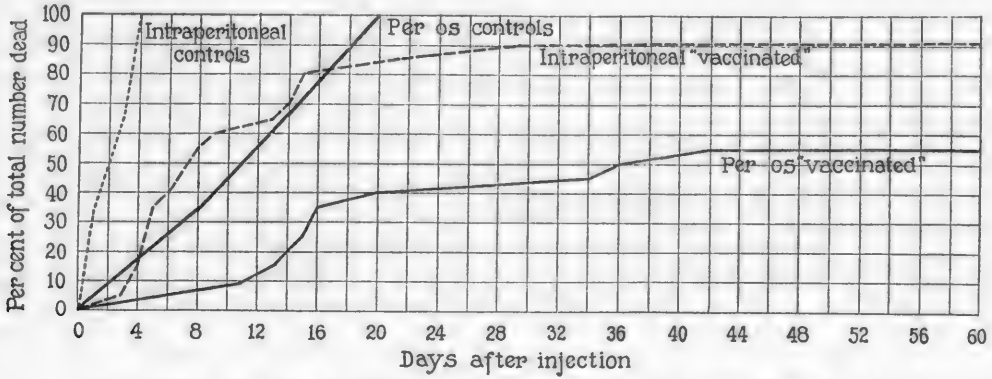
Mice.	No. of mice.	Dosage.	Duration of life.	Average duration of life.
			days	*days*
Intraperitoneal control.	3	1:100	1–3–4	2.6
" "	1	1:1,000	5	5
" "	1	1:10,000	5	5
" "vaccinated."*	20	1:100	3–4–4–5–5–5–5–6–8–8–8–9–13–14–15–15–21–29. Two survivors.†	10
Per os control.	3	1:100	8–14–20	14
" " "	1	1:1,000	Survived.†	
" " "	1	1:10,000	" †	
" "vaccinated."*	20	1:100	11–11–13–15–15–16–16–20–34–36–42. Nine survivors.†	20

* The "vaccinated" mice had received two subcutaneous injections previous to the intraperitoneal or intrastomachal injection.

† All survivors were discarded after 62 days.

The increase in partial protection following a second injection of "vaccine" is effective against the *per os* as well as the peritoneal portal of entry.

40 of the remaining "vaccinated" mice were given 25 days later a third subcutaneous injection of 250,000,000 killed bacilli, of which, after the lapse of another 10 days, 20 were injected into the peritoneum and 20 by stomach tube with the regular dose of 1 cc. of a 14 hour living broth culture diluted 1:100.



TEXT-FIG. 5. Duration of life of "vaccinated" mice injected intraperitoneally and intrastomachally. The figures are taken from Table XI.

The results are given in Table XII and differ from those in Table XI only in the number of survivors among the "vaccinated" animals, which is much larger. It must be noted, though, that the increased protection from the third "vaccination" is somewhat more effective against the introduction of live organisms intraperitoneally than by way of the mouth.

The results of these experiments show that mice "vaccinated" subcutaneously with a certain mouse typhoid strain are partially

TABLE XII.

Duration of Life of Normal and "Vaccinated" Mice Injected Intraperitoneally and Intrastomachally.

Mice.	No. of mice.	Dosage.	Duration of life.	Average duration of life.
			days	*days*
Intraperitoneal control.	3	1:100	1–1–1	1
" "	1	1:1,000	1	1
" "	1	1:10,000	5	5
" "vaccinated."*	20	1:100	7–8–8–10–10–12–12–13–15–16–17–18–32–33. Six survivors.†	15+
Per os control.	3	1:100	19–21. One survivor.†	20
" " "	1	1:1,000	Survived.†	
" " "	1	1:10,000	" †	
" " "vaccinated."*	20	1:100	8–10–10–12–18–18–21–34. Twelve survivors.†	17

* The "vaccinated" mice had received three subcutaneous injections previous to the intraperitoneal or intrastomachal injection.

† All survivors were discarded after 62 days.

protected against a subsequent introduction of the homologous live organisms intraperitoneally or *per os*, and that each dose of "vaccine" seems to confer a similar relative increase in resistance to the peritoneal or gastrointestinal route of infection. Again, given a fixed dose of this culture, it is clear that intraperitoneal injection into normal mice produces an acute type of disease, while the intrastomachal route is followed by a more chronic infection. It would appear, then, that a short interval of time is consumed by the organism in overcoming the

natural defenses of the peritoneal cavity and that a longer period of time is necessarily consumed in overcoming the natural defenses of the gastrointestinal tract, and that this time relationship is retained in immune mice.

Immunization per Os.

The experiments described bring out the nature and the limitations of the immunizing process so far as it is affected by the use of killed cultures injected into the pleural or peritoneal cavity and the subcutaneous tissues. However, the parenteral introduction of the bacilli, whether living or dead, is, as it were, a circumvention of the ordinary or natural process of infection and even perhaps of immunization. Mouse typhoid, so called, is induced by the ingestion of certain paratyphoid-enteritidis strains; and it is to be supposed that in the course of an outbreak of mouse typhoid some animals ingest the cultures, resist infection, and survive. That this is not a hypothesis is shown by the observations of Amoss,[2] by the reported studies of Topley,[4] as well as by our own experiments related above on infection *per os*.

The next step, therefore, in our experimental studies was to attempt immunization directly *per os*. Two general lines were pursued. Certain mice were fed fixed doses of the killed cultures and others were fed very minute amounts of living organisms, in both instances not once only, but over definite periods of time. Mice thus prepared were then tested for resistance by *per os* and intraperitoneal injection of living cultures in lethal doses. Protection was established by both methods, and in contrasting the results of the intrastomachal with the intraperitoneal injections, an attempt was made to determine the occurrence of a possible local gastrointestinal immunity.

Killed Cultures per Os.—The experiments to be described are somewhat intricate, but they are given in detail just because they bring out certain difficulties attending the systematic experimental investigation of mouse typhoid infections.

24 mice were fed daily for 30 days with bread soaked in a broth culture of Bacillus M. T. II, killed by heating to 55°C. for 1 hour. All the animals were

[4] Topley, W. W. C., *J. Hyg.*, 1920–21, xix, 350; *Lancet*, 1919, ii, 1.

living at the end of the feeding period, but during the next 30 days 12 died and at autopsy yielded cultures of Bacillus M. T. II. This latter point will be considered presently. The 12 survivors and 12 controls were then given by stomach tube 1 cc. of a living 1:100, 16 hour broth culture of Strain M. T. II. Of these, 1 "vaccinated" mouse died on the 12th day and yielded at autopsy a positive culture; 7 controls died within 20 days (Table XIII). The 11 survivors were then tested for blood agglutinins and for carriage of Bacillus M. T. II. The blood serum of 5 mice agglutinated the bacillus completely and of 3 mice partially in dilutions of 1:200; 6 of the 11 mice proved fecal carriers of the bacillus.

TABLE XIII.

Per Os Injection of Strain M. T. II into Mice "Vaccinated" per Os with Killed Cultures.

Mice.	No. of mice.	Duration of life in fatal cases.	No. of survivors.
		days	
"Vaccinated."	12	12	11*
Controls.	12	6–7–8–9–12–17–20	5

* "Vaccinated" survivors were used again after 37 days (see Table XIV).

TABLE XIV.

Intraperitoneal Injection of Strain M. T. II into Mice "Vaccinated" per Os with Killed Cultures.

Mice.	No. of mice.	Broth dilution.	Duration of life in fatal cases.	No. of survivors.
			days	
"Vaccinated."	11	1:500		11*
Control.	1	1:100	1	0
"	5	1:500	1–3–3–4–4	0
"	1	1:1,000	3	0
"	1	1:10,000	8	0

* Survivors were discarded after 30 days.

The 11 mice were next injected intraperitoneally with 1 cc. of a 1:500 living broth culture 16 hours old. The control mice were similarly injected with dilutions of 1:100, 1:500, 1:1,000, and 1:10,000. Table XIV presents the result of these tests. While all the control animals succumbed to this active living culture, the treated animals successfully resisted many lethal doses. Not only had the 11 mice become refractory to intrastomachal injections of living cultures which at most converted them into "carriers," but they resisted intraperitoneal injections as well.

The following interpretations of this experiment are offered. The first 12 mice which succumbed to M. T. II infection may have responded to a possible very few living bacilli contained in the "vaccine" (control plates on 3 of the 30 days showed one or two colonies) or may have been infected by the attendant who, in caring for the mice, spread living bacilli from cage to cage. Although this was noted during the epidemiological experiments of Amoss,[2] it may be stated that in our own study great precaution was taken to avoid this complication and that numbers of other normal mice held as controls at the same time and throughout the experiments did not spontaneously develop mouse typhoid. Whether the surviving "vaccinated" mice which resisted the subsequent lethal dose *per os* were protected by the repeated and large doses of killed cultures or the very few possible live organisms which they may have ingested, cannot, from this experiment, be definitely stated; the former supposition, however, is very much more probable.

Living Cultures per Os.—Experimental data as well as observations on the manner of spread of the infection in epidemics of mouse typhoid all indicate that certain mice receive the bacilli of mouse typhoid into the stomach and intestines without succumbing to the disease itself. These facts, taken together with the effect of killed cultures fed *per os* in inducing resistance to infection by living bacilli, led to the employment of minute doses of the living cultures in order to develop this refractory state.

The procedure was as follows: A 14 hour broth culture of Strain M. T. II was fed to 18 mice, so that 3 received 1 cc. each of a 1:1,000 dilution every day, 3 received the same treatment with a 1:10,000 dilution; 3 received the 1:1,000 dilution every 2nd day, 3 the 1:10,000 dilution every 2nd day; 3 the 1:1,000 and 3 the 1:10,000 dilutions every 3rd day. Feedings were discontinued after 34 days. During this period and a subsequent 27 days, all except 7 mice had succumbed to infection with Bacillus M. T. II. The 7 survivors were distributed as follows: 1 from 1:10,000 every day group; 1 from 1:1,000 every 2nd day group; 1 from 1:10,000 every 2nd day group; 2 from 1:1,000 every 3rd day group; and 2 from 1:10,000 every 3rd day group. The 7 survivors, along with 7 control mice, were now given by stomach tube 1 cc. of a 1:100 dilution of Culture M. T. II. Table XV shows the result.

The 6 surviving mice were tested (*a*) for agglutination and (*b*) for carriage of Bacillus M. T. II. The blood serum of 1 mouse agglutinated the bacilli completely in a dilution of 1:200, and 2 of the 6 proved to be fecal carriers. They

were then injected intraperitoneally with 1 cc. of a 1:500 living broth culture 16 hours old. The control mice were similarly infected with dilutions of 1:100, 1:500, 1:1,000, and 1:10,000. Table XVI summarizes the duration of life in experimental and control animals.

TABLE XV.

Per Os Injection of Strain M. T. II into Mice "Vaccinated" per Os with Live Cultures.

Mice.	No. of mice.	Duration of life in fatal cases.	No. of survivors.
		days	
"Vaccinated."	7	7*	6†
Controls.	7	5–6–6–7–9–19–36	0

* This mouse showed no pathological lesions at autopsy. Heart's blood and spleen were sterile.

† Survivors were used again after 38 days (see Table XVI).

TABLE XVI.

Intraperitoneal Injection of Strain M. T. II into Mice "Vaccinated" per Os with Live Cultures.

Mice.	No. of mice.	Broth dilution.	Duration of life in fatal cases.	No. of survivors.
			days	
"Vaccinated."	6	1:500		6*
Control.	1	1:100	1	0
"	5	1:500	1–3–3–4–4	0
"	1	1:1,000	3	0
"	1	1:10,000	8	0

* Survivors were discarded after 30 days.

The results of this experiment are of considerable interest in that they bring out the varying capacities of mice of one age and condition to accommodate to sublethal doses of a given culture of Strain M. T. II introduced into the stomach. The response to wide fluctuations of dose is particularly informing. And the experiment shows unmistakably that the initially and doubtless reinforced refractory mice may come to resist intrastomachal or intraperitoneal doses of the living culture to which all controls succumb.

That protection is afforded certain mice by successive feedings either of heat-killed or living cultures of this paratyphoid-enteritidis strain is indicated by the above experiments. The next tests were devised to bring out the mechanism of this protection—whether, for example, it is a local process confined to the gastrointestinal tract or a general phenomenon operating against the subsequent injection of organisms introduced into the peritoneal cavity as well as into the stomach.

50 mice were tested for blood agglutinins in serum dilutions of 1:20 and 1:200. All were negative. Stool cultures of these mice showed no organisms of the paratyphoid-enteritidis group. "Vaccine" prepared as follows was then added

TABLE XVII.

Intraperitoneal and per Os Injection of Strain M. T. II into Mice "Vaccinated" per Os with Killed Cultures.

Mice.	No. of mice.	Broth dilution.	Duration of life in fatal cases.	No. of survivors.
			days	
Intraperitoneal controls.	4	1:100	1–2–3–3	0
" "	1	1:1,000	8	0
" "	1	1:10,000	9	0
" "	1	1:100,000	7	0
" "vaccinated."	16	1:100	4–4–4–4–4–6–6–6–8–8–9–16	4*
Per os controls.	1	1:10	7	0
" " "	4	1:100	9–10–50	1
" " "vaccinated."	17	1:100	5–6–7–11–12–16–17–25–31–51	7

* Survivors were discarded after 70 days.

daily to the feedings. 10 cc. of a 24 hour broth culture of Strain M. T. II, heated at 55°C. for 2 hours, were added to 40 cc. of milk, soaked up with bread, and divided roughly into ten parts so that each jar of 5 mice received approximately 1 cc. of the original "vaccine." This relatively small dose was continued over a period of 4 weeks. After an interval of 1 week, when it was seen that all the mice were in good condition, agglutination tests were again set up and stool cultures were taken, with negative results.

16 of these mice were then given intraperitoneally 1 cc. of a 1:100 dilution of a 24 hour broth culture of Strain M. T. II; 17 mice received the same dosage *per os*. Controls were injected intraperitoneally and *per os* with doses from 1:10 to 1:100,000. The duration of life in the series is analyzed in Table XVII and Text-fig. 6. Intraperitoneal injection was lethal in a dilution of 1:100,000; the intraperitoneal controls were dead in 3 days. Of the "vaccinated" mice

receiving the intraperitoneal injection, 30 per cent died on the 4th day, 75 per cent were dead on the 16th day, and 25 per cent survived over a period of 10 weeks. The animals receiving live organisms *per os* were somewhat irregular; 80 per cent of the controls succumbed, while only 55 per cent of the "vaccinated" mice died within a period of 10 weeks.

In this experiment we have established, therefore, as a response to the continued ingestion of killed organisms in small doses, a partial protection against live organisms introduced by mouth or by way of the peritoneal cavity, and this protection is in the nature of a general rather than a local immunity.

Finally, to show the general nature of the protection in a more striking manner, immunization was carried out with live organisms.

TABLE XVIII.

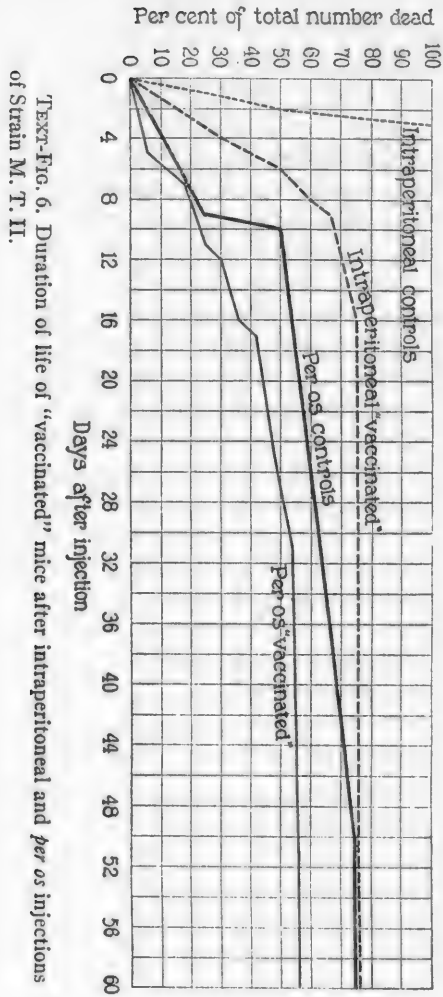
Intraperitoneal Injection of Strain M. T. II into Mice "Vaccinated" per Os with Live Cultures.

Mice.	No. of mice.	Broth dilution.	Duration of life in fatal cases.	No. of survivors.
			days	
Experimental.	18	1:1,000	2–6–12–16	14*
Controls.	1	1:100	1	0
"	7	1:1,000	1–1–1–1–2–2–4	0
"	1	1:10,000	3	0

* Survivors were discarded after 30 days.

25 mice were tested for blood agglutinins for Bacillus M. T. II with serum dilutions of 1:20 and 1:200. None of the mice reacted. Daily feedings of approximately 1 cc. per mouse were now begun with a 16 hour living broth culture of Strain M. T. II diluted 1:10,000 in milk. The total quantities were as follows: 5 mice received 5, 5 received 7, 5 received 9, 5 received 11, and 5 received 13 feedings. Within a 20 day period, 6 of the fed mice succumbed to mouse typhoid infection. The second test for agglutinins was made 13 days after the feedings began and none were found. 23 days later, or 36 days after the experiment began, of 18 surviving mice, 7 showed blood agglutinins in 1:200 dilution. Only 1 mouse was determined to be an M. T. II bacillus carrier. This experiment was terminated by inoculating intraperitoneally the entire 18 survivors of the feeding series along with controls with 1 cc. of a 1:1,000 dilution of an 8 hour living broth culture of Strain M. T. II. Table XVIII shows the result.

While all the controls died, 14 of the fed mice survived. None of the 4 fatalities had previously shown blood agglutinins. It may be



TEXT-FIG. 6. Duration of life of "vaccinated" mice after intraperitoneal and *per os* injections of Strain M. T. II.

seen, therefore, that mice which ingest living cultures of Strain M. T. II and survive may develop a general immunity accompanied in a certain number of cases by blood agglutinins.

DISCUSSION.

As part of a more general study of mouse typhoid infection the experiments in this paper relate to the manner of interaction between the mouse and a native pathogen belonging to the paratyphoid-enteritidis group. The results may be stated in summary about as follows:

Among any considerable number of healthy mice of a given age and size a small number will prove refractory to inoculation with a minimum lethal dose of a mouse strain of *Bacillus pestis caviæ*, no matter into what part of the body the proper injection is made. Minimum lethal dosage is, therefore, a highly relative matter.

Taking the far greater part of the mice chosen for experiment, however, the duration of life and type of infection depend on the dose, the site of injection, and on the individual resistance of the animals as ordinarily present or as artificially produced through immunization. Lesions found at autopsy depend somewhat upon the site of the injection, but chiefly upon the duration of life after the inoculation.

When fixed doses of this organism are injected into the pleura and peritoneum of normal mice, there occurs first an initial period of lag in bacterial growth, during which time—4 to 6 hours—the number of bacilli decreases; subsequently the bacilli multiply rapidly until death occurs, usually within 8 days. This demonstration *in vivo* of bacterial lag parallels in a rough manner the careful work of Penfold,[5] Chesney,[6] and Graham-Smith[7] on bacterial growth *in vitro*.

When mice are "vaccinated" with dead bacilli into the pleural or peritoneal cavity or even subcutaneously, the process of infection is altered. Live bacilli injected into the pleural and peritoneal cavities of such animals rapidly diminish in number and may apparently disappear entirely from the exudate. But usually after remaining at a low level for 2 or 3 days, they gradually begin to increase and continue a slow rise until death ensues, within about 18 days. Accord-

[5] Penfold, W. J., *J. Hyg.*, 1914, xiv, 215.
[6] Chesney, A. M., *J. Exp. Med.*, 1916, xxiv, 387.
[7] Graham-Smith, G. S., *J. Hyg.*, 1920–21, xix, 133.

ing to degree of "vaccination" the subsequent inoculation of live cultures may produce death only after a greater time period or may fail to kill.

Paul Ehrlich was probably the first to demonstrate the possibility of immunization by the gastrointestinal route.[8] By feeding ricin and abrin food cakes to mice, the animals not only developed a tolerance to the feeding of several lethal doses but resisted 400 lethal doses injected subcutaneously. Loeffler,[9] Wolf,[10] Yoshida,[11] and Brückner,[12] by feeding killed or living cultures of certain animal paratyphoid strains to mice, have established a definite immunity against the subsequent feeding in large doses of the homologous live organism. The controversy throughout this work as to whether the induced gastrointestinal immunity is local or general has been renewed by the recent work of Besredka with dysentery and paratyphoid bacilli on mice and rabbits.[13] He offers the somewhat startling hypothesis that not only is the gastrointestinal immunity entirely of a local nature, but that any mode of vaccination is followed by only one type of immunity—local: "Tout comme l'immunité naturelle, l'immunité artificielle vis-à-vis des virus typhique et paratyhique repose sur celle de la paroi intestinale: elle est d'essence locale."

Throughout our work there has been no evidence of acquired local immunity as opposed to a condition of general immunity. The feeding of killed or living cultures of a mouse typhoid strain protects the mice against lethal doses of living bacilli injected *per os* or intraperitoneally. And when killed bacilli are injected into the pleura or peritoneum, those cavities are rendered no more resistant to the introduction of the living bacilli than when the "vaccination" is made subcutaneously. It seems, therefore, that protective principles following the introduction of killed or living bacilli into any part of the body operate against the later injection of living organisms, regardless of their portal of entry.

[8] Ehrlich, P., *Deutsch. med. Woch.*, 1891, xvii, 976, 1218.

[9] Loeffler, F., *Gdnkschr. verstorb. Generalstabsarzt Armee . . . . v. Leuthold*, 1906, i, 249.

[10] Wolf, K., *Münch. med. Woch.*, 1908, lv, 270.

[11] Yoshida, E., *Arch. Hyg.*, 1909, lxix, 21.

[12] Brückner, G., *Z. Immunitätsforsch., Orig.*, 1910–11, viii, 439.

[13] Besredka, A., and Basseches, S., *Ann. Inst. Pasteur*, 1918, xxxii, 193. Besredka, A., *Ann. Inst. Pasteur*, 1919, xxxiii, 301, 557, 882.

A word should be said concerning experimental infection *per os*, the normal portal of entry. Whereas earlier writers have experienced difficulty in producing the disease in this manner, we have found it possible, within certain limits, to infect regularly. These limits, however, are subject to such fluctuations that results must be interpreted with caution. In these experiments two facts must be remembered: first, that all tests were conducted in large battery jars containing either 3 or 6 mice and that this constant contact factor must have raised the per cent of mortality in controls as well as experimental animals; secondly, that only wide deviations in mortality curves were considered as significant.

SUMMARY.

If live cultures of a mouse strain of *Bacillus pestis caviæ* are injected intrapleurally or intraperitoneally into normal mice, there occurs an initial lag in the rate of bacterial multiplication lasting a few hours, followed by a rapid and continued acceleration of growth until the death of the animal.

If live cultures of this organism are given *per os* to normal mice, there occurs an incubation period of 5 to 6 days, after which the animal usually develops symptoms of disease and succumbs. A small percentage of mice, however, proves refractory to infection by this route.

If live cultures of this organism are injected intrapleurally or intraperitoneally into mice previously "vaccinated" intrapleurally or intraperitoneally, they are partially destroyed and held in check by the protective mechanisms of the animal body for 2 or 3 days. Subsequently the rate of bacterial multiplication increases gradually until the death of the animal. The partial protection following this type of "vaccination" is entirely of a general nature; no evidence of a local immunity has been obtained.

Mice given one, two, or three subcutaneous doses of "vaccine" show a similar relative increase in resistance to the subsequent intraperitoneal or *per os* injection of live organisms.

Feeding mice live or killed cultures of this organism induces a definite protection against subsequent intrastomachal and intraperitoneal injections of live organisms. The immunity developed in this way is also of a general as opposed to a local nature.

IDENTIFICATION OF A PARATYPHOID-ENTERITIDIS STRAIN ASSOCIATED WITH EPIZOOTICS OF MOUSE TYPHOID.*

By LESLIE T. WEBSTER, M.D.

(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

(Received for publication, April 5, 1922.)

In other papers of this series, Amoss[1] and Lynch[2] have described epizootics of mouse typhoid associated with a bacillus of the paratyphoid-enteritidis group, and with this same organism, designated as Strain M. T. II, the author has studied certain corollary protection and immunity phenomena.[3] It seems important, therefore, to classify definitely this M. T. II strain and to relate it as far as possible to other similar pathogens.

B. typhi murium, isolated by Loeffler[4] from an epizootic of laboratory mice and related to the colon-typhoid group, was considered by him to be the causative microbe of mouse typhoid. In France this etiological rôle was assigned to a similar organism, Danysz' bacillus, recovered from a plague of field mice.[5] Later serological studies of Bainbridge[6] on the paratyphoid and food poisoning bacilli indicate that Danysz' bacillus is identical with *B. enteritidis* Gärtner and that *B. typhi murium* does not exist as an entity since some strains correspond to

---

* In a communication to the Society for Experimental Biology and Medicine (Webster, L. T., *Proc. Soc. Exp. Biol. and Med.*, 1921–22, xix, 71) this organism was designated as *B. enteritidis* (*murium*).

[1] Amoss, H. L., *J. Exp. Med.*, 1922, xxxvi, 25.

[2] Lynch, C. J., *J. Exp. Med.*, 1922, xxxvi, 15.

[3] Webster, L. T., *J. Exp. Med.*, 1922, xxxvi, 71.

[4] Loeffler, F., *Centr. Bakt.*, 1892, xi, 129.

[5] Danysz, J., *Ann. Inst. Pasteur*, 1900, xiv, 193.

[6] Bainbridge, F. A., *J. Path. and Bact.*, 1909, xiii, 443.

B. enteritidis and others to *B. aertrycke*.[7] Krumwiede[8] has found that strains of so called *B. typhi murium* belong either to the enteritidis group or to a class of paratyphoid-like strains of rodent origin (*B. pestis caviæ*.)[9] Finally, throughout Topley's study of an experimental mouse epizootic induced by the feeding of *B. enteritidis*, his autopsy protocols note the recovery of *B. enteritidis* and *B. aertrycke*.[7,10] Apparently, then, *B. typhi murium* has no significance as an entity; Danysz' bacillus is synonymous with *B. enteritidis* Gärtner; and the microbes usually associated with mouse typhoid are *B. enteritidis* Gärtner, *B. aertrycke*, and *B. pestis caviæ* Smith.

Morphological and Cultural Characteristics.

Strain M. T. II is a Gram-negative, motile, non-sporulating bacillus. Agar colonies are thin, bluish, and somewhat translucent, with irregular edges. Dextrose, levulose, maltose, mannitol, xylose, arabinose, rhamnose, and inosite are fermented. Gas is formed, milk is not coagulated, indole is not produced, lead acetate medium is blackened.

These characteristics place the organism in the paratyphoid-enteritidis group. The fermentation of arabinose and the reaction in lead acetate medium would tend to exclude *Bacillus suipestifer*, while the vigorous production of acid in inosite eliminates *Bacillus enteritidis* and favors the paratyphoid group. However, it is necessary to correlate these observations with the more accurate information derived from serological tests.

Serological Studies.

An immune rabbit serum was prepared from Strain M. T. II. Stock cultures of the paratyphoid-enteritidis group were seeded into plain broth, pH 7.4, incubated for 24 hours, and mixed with equal parts of 0.2 per cent formalin in 0.85 per cent salt solution. Agglutination tubes were set up to contain one part of serum dilution to nine

[7] Topley, Weir, and Wilson (Topley, W. W. C., Weir, H. B., and Wilson, G. S., *J. Hyg.*, 1921, xx, 241) make the following statement in regard to paratyphoid bacilli of animal origin: "It is probable that *B. aertrycke* belongs to this subgroup, and also those *B. suipestifer* strains of German origin which were studied by Bainbridge and O'Brien (1911). The bacilli isolated from mice dying during our own experiments, and hitherto referred to as *B. suipestifer*, have all the characteristics of this subgroup and should be placed in it."

[8] Krumwiede, C., Jr., Valentine, E., and Kohn, L. A., *J. Med. Research*, 1918–19, xxxix, 449.

[9] Wherry, W. B., *J. Infect. Dis.*, 1908, v, 519.

[10] Topley, W. W. C., *J. Hyg.*, 1920–21, xix, 350.

parts of formalinized antigen. The several strains employed may be described as follows:

M. T. II. Unknown strain for identification.
B. pestis caviæ, No. 146. (*Ferry 63*).[8]
320 mutton type. "*Aertrycke*" strain from Schütze;[11] called by him "Mutton type Calf 6."
B. choleræ suis, No. 350 (*Smith*). Isolated by TenBroeck in 1919.
B. paratyphosus B. No. 178 Army strain.
313 Stanley. "*Aertrycke*" strain from Schütze; Hutchins.[11]
315 G. "*Aertrycke*" strain from Schütze; monkey.[11]
316 Reading. "*Aertrycke*" strain from Schütze; water supply.[11]
317 Newport. "*Aertrycke*" strain from Schütze; Fentry strain; isolated by Perry.[11]
B. enteritidis, No. 47. Gärtner.
B. enteritidis, No. 273. McWeeny strain; from Jordan.
B. abortus equi, 215 Meyer No. 7. Kentucky.
B. paratyphosus A. Army strain.

From Table I it may be seen that while Serum M. T. II fails to agglutinate *Bacillus enteritidis*, *Bacillus abortus equi*, and *Bacillus paratyphosus* A, and while it reacts only slightly with the Army strain of *Bacillus paratyphosus* B, *Bacillus choleræ suis*, and the Stanley, G, Reading, and Newport *aertrycke* strains of Schütze, it does agglutinate the "mutton" strain of Schütze and *Bacillus pestis caviæ* to a titer approximating that of the homologous strain.

To emphasize this relationship, sera of the various type strains were set up against Strain M. T. II (Table II).

This cross-agglutination eliminates the Army Para B, *Bacillus choleræ suis*, and the Stanley, G, Reading, and Newport strains. "Mutton" serum, however, agglutinates Strain M. T. II to nearly as high a titer as the homologous strain and *Bacillus pestis caviæ* serum agglutinates Strain M. T. II and homologous strain to equal titer.

Final evidence relating Strain M. T. II to *Bacillus pestis caviæ* and the "mutton" strain was obtained by absorption tests.

Some quantitative measurement of absorbing antigen is necessary for accurate interpretation of absorption phenomena. In these tests,

[11] Schütze, H., *Lancet*, 1920, i, 93.

therefore, a constant ratio between each serum volume and the volume of the absorbing strain was determined by measuring the volume of packed cell antigen and then adding the calculated amount of serum diluted 1:10. For the low titer "mutton" serum this ratio was 1:4; for the *Bacillus pestis caviæ* serum a 1:2 ratio was employed, while in the case of Serum M. T. II, it was necessary to absorb twice with a ratio of packed cells to serum of 1:3. For controls each absorption

TABLE I.

Agglutination with Serum M. T. II against Various Strains.

Strain.	Serum M. T. II dilution.							
	1:100	1:1,000	1:5,000	1:7,000	1:9,000	1:10,000	1:20,000	1:50,000
M. T. II.	++	++	++	++	++	++	++	−
B. pestis caviæ, No. 146.	++	+1	+1	+1	+1	+1	+	−
320 mutton.	++	++	++	+1	+1	+1	+	−
B. choleræ suis, No. 350 (Smith).	+1	+1	−	−	−	−	−	−
Para B Army.	+1	+1	−	−	−	−	−	−
313 Stanley.	+1	+	−	−	−	−	−	−
315 G.	+1	+1	−	−	−	−	−	−
316 Reading.	+1	+1	−	−	−	−	−	−
317 Newport.	+	+	−	−	−	−	−	−
B. enteritidis, No. 47.	−	−	−	−	−	−	−	−
" " " 273.	−	−	−	−	−	−	−	−
" *abortus equi*.	−	−	−	−	−	−	−	−
Para A Army.	−	−	−	−	−	−	−	−

++ indicates complete agglutination; +1, marked agglutination; +, slight agglutination; 1, trace of agglutination.

test was paralleled by agglutination of unabsorbed serum with homologous and heterologous strains and by absorption and subsequent agglutination with the homologous strain. The results are presented in Tables III to VI.

Table III shows that Strain M. T. II completely absorbed its own agglutinins from "mutton" serum and 98 per cent of the homologous agglutinins. Conversely (Table IV), the "mutton" strain, after one absorption, removed 90 per cent of its own agglutinins from Serum M. T. II and 75 per cent of the homologous agglutinins, and, after

two absorptions, the "mutton" strain removed all of its own agglutinins and 93 per cent of the homologous agglutinins.

It is probable then, that Strain M. T. II and the "mutton" strain are antigenically very similar but not quite identical.

TABLE II.

Direct Agglutination with Various Sera against Strain M. T. II.

Serum.	Strain.	Serum dilution.										
		1:100	1:200	1:400	1:800	1:1,600	1:3,200	1:6,400	1:12,800	1:25,600	1:51,200	1:102,400	Control.
B. pestis caviæ.	*B. pestis caviæ.*	++	++	++	++	++	++	+	1	−	−	−	−
	M. T. II.	++	++	++	++	++	++	+	1	−	−	−	−
"Mutton."	"Mutton."	++	++	++	++	++	+1	1	1	−	−	−	−
	M. T. II.	++	++	++	++	++	1	−	−	−	−	−	−
B. choleræ suis, No. 350.	*B. choleræ suis,* No. 350.	++	++	++	++	++	++	+1	+	+	1	−	−
	M. T. II.	−	−	−	−	−	−	−	−	−	−	−	−
B. paratyphosus B, No. 178.	*B. paratyphosus* B.	++	++	++	++	++	++	+1	+	+	1	−	−
	M. T. II.	−	−	−	−	−	−	−	−	−	−	−	−
313 Stanley.	313 Stanley.	++	++	++	++	++	+	1	−	−	−	−	−
	M. T. II.	−	−	−	−	−	−	−	−	−	−	−	−
315 G.	315 G.	++	++	++	++	++	++	+	+	1	−	−	−
	M. T. II.	−	−	−	−	−	−	−	−	−	−	−	−
316 Reading.	316 Reading.	++	++	++	++	++	++	+	−	−	−	−	−
	M. T. II.	−	−	−	−	−	−	−	−	−	−	−	−
317 Newport.	317 Newport.	++	++	++	++	++	+	−	−	−	−	−	−
	M. T. II.	−	−	−	−	−	−	−	−	−	−	−	−

Table V shows that Strain M. T. II absorbed 97 per cent of its own agglutinins from *Bacillus pestis caviæ* serum and 97 per cent of the homologous agglutinins. Conversely (Table VI), the *Bacillus pestis caviæ* strain after one absorption removed 98 per cent of its own agglutinins from Serum M. T. II and 98 per cent of the ho-

TABLE III.

"Mutton" Serum.

Strain.	Unabsorbed serum.												Serum absorbed by "mutton" strain.												Serum absorbed by Strain M. T. II.											
	1:100	1:200	1:400	1:800	1:1,600	1:3,200	1:6,400	1:12,800	1:25,600	1:51,200	1:102,400	Control.	1:50	1:100	1:200	1:400	1:800	1:1,600	1:3,200	1:6,400	1:12,800	1:25,600	1:51,200		1:50	1:100	1:200	1:400	1:800	1:1,600	1:3,200	1:6,400	1:12,800	1:25,600	1:51,200	
"Mutton."	++	++	++	++	++	+	1	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	1	−	−	−	−	−	−	−	−	−	−
M. T. II	++	++	++	++	+	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−

TABLE IV.

Serum M. T. II.

Strain.	Unabsorbed serum.												Single absorption by Strain M. T. II.												Single absorption by "mutton" strain.											
	1:100	1:200	1:400	1:800	1:1,600	1:3,200	1:6,400	1:12,800	1:25,600	1:51,200	1:102,400	Control.	1:50	1:100	1:200	1:400	1:800	1:1,600	1:3,200	1:6,400	1:12,800	1:25,600	1:51,200	1:102,400	1:50	1:100	1:200	1:400	1:800	1:1,600	1:3,200	1:6,400	1:12,800	1:25,600	1:51,200	1:102,400
M. T. II..	++	++	++	++	++	++	++	++	+1	1	−	−	++	++	++	+1	+	1	−	−	−	−	−	−	++	++	++	++	++	++	++	+1	+	−	−	−
"Mutton."	++	++	++	++	++	++	++	+1	+	1	−	−													++	++	++	++	+1	+1	+	−	−	−	−	−

Strain.	Double absorption by Strain M. T. II.										Double absorption by "mutton" strain.									
	1:50	1:100	1:200	1:400	1:800	1:1,600	1:3,200	1:6,400	1:12,800	1:25,600	1:51,200	1:50	1:100	1:200	1:400	1:800	1:1,600	1:3,200	1:6,400	1:12,800	1:25,600	1:51,200
M. T. II	−	−	−	−	−	−	−	−	−	−	−	+1	+1	+1	+1	+1	+1	−	−	−	−	−
"Mutton."												−	−	−	−	−	−	−	−	−	−	−

TABLE V.

B. pestis caviæ Serum.

Strain.	Unabsorbed serum.												Serum absorbed by *B. pestis caviæ*.												Serum absorbed by Strain M. T. II.											
	1:100	1:200	1:400	1:800	1:1,600	1:3,200	1:6,400	1:12,800	1:25,600	1:51,200	1:102,400	Control.	1:50	1:100	1:200	1:400	1:800	1:1,600	1:3,200	1:6,400	1:12,800	1:25,600	1:51,200		1:50	1:100	1:200	1:400	1:800	1:1,600	1:3,200	1:6,400	1:12,800	1:25,600	1:51,200	
B. pestis caviæ...	++	++	++	++	++	++	+1	+	1	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	+	+	+	+	+	—	—	—	—	—	—
M. T. II.....	++	++	++	++	++	++	++	+1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+	+	+	—	—	—	—	—	—

TABLE VI.

Serum M. T. II.

Strain.	Unabsorbed serum.												Single absorption by Strain M. T. II.												Single absorption by *B. pestis caviæ*.											
	1:100	1:200	1:400	1:800	1:1,600	1:3,200	1:6,400	1:12,800	1:25,600	1:51,200	1:102,400	Control.	1:50	1:100	1:200	1:400	1:800	1:1,600	1:3,200	1:6,400	1:12,800	1:25,600	1:51,200	1:102,400	1:50	1:100	1:200	1:400	1:800	1:1,600	1:3,200	1:6,400	1:12,800	1:25,600	1:51,200	1:102,400
M. T. II..	++	++	++	++	++	++	++	++	+1	1	—	—	++	++	++	+1	+	1	—	—	—	—	—	—	++	++	++	++	+	1	—	—	—	—	—	—
B. pestis...																																				
caviæ....	++	++	++	++	++	++	++	+1	+1	1	—	—		+1	+	+	+	—	—	—	—	—	—	—	++	++	++	+1	+	—	—	—	—	—	—	—

Strain.	Double absorption by Strain M. T. II.											Double absorption by *B. pestis caviæ*.										
	1:50	1:100	1:200	1:400	1:800	1:1,600	1:3,200	1:6,400	1:12,800	1:25,600	1:51,200	1:50	1:100	1:200	1:400	1:800	1:1,600	1:3,200	1:6,400	1:12,800	1:25,600	1:51,200
M. T. II.....	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
B. pestis caviæ.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

mologous agglutinins, and, after two absorptions, the *Bacillus pestis caviæ* strain removed all of its own agglutinins and all of the homologous agglutinins from Serum M. T. II.

Clearly, then, Strain M. T. II and the type strain *Bacillus pestis caviæ* are antigenically identical.

DISCUSSION.

The "mutton" group forms the chief division in Schütze's serological study of the paratyphoid *aertrycke* types.[11] He says: "The Mutton group claims over 50 per cent of the strains, and would appear to be the most important; it includes strains from various countries and from animal as well as human epidemics."

The *Bacillus pestis caviæ* group, "a distinct paratyphoid type or group of bacilli, is encountered therefore in spontaneous infections in laboratory animals, especially rodents" according to Krumwiede.[8] Thirteen guinea pigs, two mice, one cat, and one rabbit strains were found by him to be antigenically similar.

Strain M. T. II is apparently similar to the type "mutton" strain of Schütze and is identical with the type *Bacillus pestis caviæ* strain.[12]

To have closely related this mouse typhoid strain to other paratyphoid types is to emphasize once again the ubiquity of the paratyphoid group and the possibility that the various strains found in mice, rats, guinea pigs, sheep, and doubtless in other domestic animals, active, capable of producing epizootics, may likewise be human pathogens with greater or less degree of virulence. Precise information concerning the underlying principles of mouse typhoid infection and epizootics should, therefore, be of great service in the interpretation of similar phenomena of man.

CONCLUSIONS.

A bacillus of the paratyphoid-enteritidis group associated with epizootics among laboratory mice has been identified with *Bacillus pestis caviæ* Smith which produces similar affections in guinea pigs and has been very closely related to the type "mutton" *aertrycke* strain of Schütze.

[12] Unpublished studies of Krumwiede and Cooper have already demonstrated a close relation between the *B. pestis caviæ* group and the "mutton" types of Schütze.

This identification is based upon the cultural reactions of the organism, direct and cross-agglutinations, and similar absorptive capacities of the unknown and type strains.

The author wishes to thank Dr. Charles Krumwiede and Miss Georgia Cooper for the type strains and type antisera and for assistance especially as regards the technical details of the absorption method which they employ.

IMMUNOLOGICAL DISTINCTIONS OF TWO STRAINS OF THE MOUSE TYPHOID GROUP ISOLATED DURING TWO SPONTANEOUS OUTBREAKS AMONG THE SAME STOCK.

By HAROLD L. AMOSS, M.D., and PETER P. HASELBAUER.
(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

(Received for publication, April 6, 1922.)

In the cancer breeding station maintained at The Rockefeller Institute there occurred, during 2½ years, two separate outbreaks of mouse typhoid among the 2,500 to 4,000 mice. The first of these appeared in the autumn of 1918 and the second exactly 2 years later. They are described by Lynch[1] in a separate paper of this series.

From the spleens of mice dying in the first outbreak there were isolated several strains which belonged to the paratyphoid-enteritidis group. One of them, Mouse·Typhoid I, was selected for use in inciting an experimental epidemic, but before starting the experiments, this strain was passed through four mice *per os* in order to assure its pathogenic power. The strain, recovered from the fourth mouse, was used in a series of artificially produced epidemics among mice for more than 2 years. Meanwhile, antiserum had been prepared against Mouse Typhoid I, and while active in high dilution against the homologous strain, it did not agglutinate the unidentified strain. It was evident that the two strains, both belonging to the paratyphoid-enteritidis group as determined by fermentation tests, were not identical. Antiserum against the new strain was prepared, but it did not agglutinate Mouse Typhoid I. This serum, however, did agglutinate the strains isolated from the spleens of mice dying in the experimental epidemic, and the inference is that the unidentified strain (now called Mouse Typhoid II) was the organism concerned in the epidemic, for no members of Mouse Typhoid I type were obtained.

In the preliminary feeding experiment to which reference has been made, Mouse Typhoid I was employed, but Mouse Typhoid II was recovered from the spleen of the fourth mouse in the series. The

[1] Lynch, C. J., *J. Exp. Med.*, 1922, xxxvi, 15.

probable explanation is that one of the mice used in the experiment was already infected with Mouse Typhoid II. This view is strengthened by the fact that occasionally among the stock from which these mice were taken deaths occurred and from the spleens only Mouse Typhoid II was isolated. Moreover, in the second epidemic among the cancer mice occurring in the autumn of 1920, Mouse Typhoid II was regularly obtained.

EXPERIMENTAL.

Agglutination Tests.

Monovalent sera were prepared by immunizing rabbits against Mouse Typhoid I, Mouse Typhoid II, and Paratyphoid B Rowland.[2]

TABLE I.

Agglutination of Various Strains of the Paratyphoid-Enteritidis Group in Three Monovalent Antisera.

Antigen.	Highest dilution showing complete agglutination. Serum.		
	Mouse Typhoid I.	Mouse Typhoid II.	Paratyphoid B Rowland.
Mouse Typhoid I	1:2,000	0	0
" " II	0	1:1,500	1:800
Paratyphoid B Rowland (Army Medical School)	0	1:800	1:1,200
Paratyphoid B Black (Army Medical School).	0	1:1,000	1:1,200
" " Kendall I	0	1:1,000	1:4,000
" " " II	0	1:1,000	1:5,000
Enteritidis—Kendall	1:2,000	0	0
" 18 (American Museum of Natural History)	0	0	0
Enteritidis 273 Gärtner	1:800	0	0
Animal typhoid—Smith.			
Mouse 2. Rat. Guinea pig. Dog 1.	>1:400*	0	0
Calf.	0	>1:400*	0

* Higher dilution not made.

[2] Paratyphoid B Rowland is the strain used by the Army Medical School in the preparation of the triple typhoid vaccine.

The results of the agglutination tests with these strains and enteritidis strains and other paratyphoid B strains[3] are shown in Table I.

It is evident that Mouse Typhoid II is closely allied antigenically to the human paratyphoid B strains tested, while Mouse Typhoid I is totally different. Mouse Typhoid I is related to the enteritidis strains Kendall and Gärtner 273 but not to the culture obtained from the American Museum of Natural History.

Animal Strains.

The following animal typhoid strains[4] were roughly classified by agglutination in Mouse Typhoid I and Mouse Typhoid II serum in a dilution of 1:400, as follows: Mouse 2, rat, guinea pig, and Dog 1 strains were completely agglutinated by Mouse Typhoid I serum in 1:400 and not by Mouse Typhoid II serum; while the calf strain was completely agglutinated by Mouse Typhoid II serum and not by Mouse Typhoid I serum.

Absorption Tests.

Mouse Typhoid II and Para B Sera.

Antigen for absorption was prepared by washing 16 hour growths from slant agar pH 7.4 with isotonic salt solution, filtering through cotton wool, and standardizing in a turbidimeter. The absorptions with the same amount of antigen in every case were made four times during 3 hours at 37°C. in dilutions 1:10, 1:20, 1:25, and 1:50 respectively. The results of agglutination tests on the Mouse Typhoid II and Para B sera after absorption are given in Table II.

It appears from these tables that Mouse Typhoid II and Para B are not identical. Para B Rowland and Black strains absorb agglutinins against themselves from Mouse Typhoid II serum, but leave some Mouse Typhoid II agglutinins. Para B serum is absorbed more slowly even by the homologous strain. The difference between Mouse Typhoid II and Para B Rowland and Black is also

[3] Strains of paratyphoid B and enteritidis were obtained from the Army Medical School, Dr. A. I. Kendall, and the American Museum of Natural History.

[4] These strains were furnished by Dr. Theobald Smith.

TABLE II.

Agglutination after Absorption.

Test strain.	Before absorption.	Absorbing Strain.					
		Mouse Typhoid II.	Para B Rowland.	Para B Black.	Para B Kendall I.*	Para B Kendall II.*	Mouse Typhoid I.
Mouse Typhoid II serum.							
Mouse Typhoid II	1:1,500	0	++1:400	++1:500	+1:200 ++1:100	+1:300 +1:200	+++1:1,500
Para B Rowland	1:800	0	0	0	+1:200 ++1:100	0	—
" " Black	1:1,000	0	0	0	+1:200 ++1:100	0	—
" " Kendall I.	1:1,000	++1:100	++1:200	++1:100, 1:200	++1:400 +++1:300	++1:400 +++1:300	—
" " " II	1:1,000	++1:100	++1:200	+++1:100	++1:400 +++1:300	++1:200	—
Calf typhoid	1:400†	0	—	—	—	—	—
Para B Rowland serum. Regular technique four times in 3 hrs.							
Mouse Typhoid II	1:800	+1:100	+1:100	+1:100	+++1:500	+++1:500	—
Para B Rowland	1:1,200	++1:400 +++1:100	+1:300 +1:100	++1:100	+++1:500	+++1:800	—
" " Black	1:1,200	++1:300 +++1:100	++1:300 ++1:100	++1:200 ++1:100	+++1:500	+++1:800	—
" " Kendall I.	1:4,000	+++1:1,000	+++1:500	+++1:500	+++1:1,000 ++1:2,000	+++1:2,000	—
" " " II	1:5,000	+++1:2,000	+++1:800	+++1:500	+1:2,000 ++1:1,000	+++1:2,000	—

Para B Rowland serum. Regular technique eight times in 16 hrs.

Mouse Typhoid II	1:800	0	0	0	0	0	—
Para B Rowland	1:1,200	+1:300 ++1:100	0	0	0	0	—
" " Black	1:1,200	+1:300 ++1:100	0	0	0	0	—
" " Kendall I	1:4,000	++1:400 +++1:300	+1:100	+1:200 +1:100	0	0	—
" " " II	1:5,000	++1:400 +++1:300	+1:200	+1:200 ++1:100	0	0	—

+++ indicates complete agglutination, clear above; ++, marked clumping, turbid above; +, definite clumping; 0, no agglutination; —, test not made.

* Absorbed five times.

† Higher dilutions not made.

shown after absorption eight times over 16 hours at 37°C., when Mouse Typhoid II removes all agglutinins reacting against it but leaves agglutinins for Para B strains.

Para B Kendall I and II are still slower in absorbing and are not identical with Para B Rowland.

Mouse Typhoid I has no power of absorption in Mouse Typhoid II serum.

Mouse Typhoid I Serum.

By direct agglutination Mouse Typhoid I seems to be related to four animal typhoid strains and also to Enteritidis Kendall and

TABLE III.

Agglutination after Absorption.

Test strain.	Before absorption.	Absorbing strains.			
		Mouse Typhoid I.	Enteritidis 18.	Enteritidis Kendall.	Enteritidis 273.
Mouse Typhoid I.....	1:2,000	0	+1:1,000 +++1:500	+1:800 +++1:400	+1:1,000 ++1:200
Enteritidis 18.....	0	0	0	0	
" Kendall.....	1:2,000	0	+1:1,000 +++1:400	0	—
" 273.....	1:800	0	—	—	0
Mouse Typhoid 2 (Smith)..	>1:400	0	—	—	0
Rat typhoid (Smith).....	>1:400	0	—	—	0
Guinea pig typhoid (Smith)	>1:400	0	—	—	+1:300
Dog Typhoid 1 (Smith)....	>1:400	0	—	—	+1:800

Enteritidis 273 Gärtner, but the latter are differentiated by absorption with the method already described.

Table III shows that Mouse Typhoid I removes all agglutinins from Mouse Typhoid I serum, but Enteritidis Kendall and 273 absorb all agglutinins against themselves from Mouse Typhoid I serum, but not all which are active against Mouse Typhoid I. The fact that Enteritidis 18, though not agglutinated in Mouse Typhoid I serum, apparently absorbs some of the agglutinins, is perhaps an example of non-specific or physical adsorption.

SUMMARY.

Two strains of the paratyphoid B-enteritidis group causing separate epidemics of mouse typhoid among 2,500 to 4,000 cancer breeding mice are found to be antigenically different. Mouse Typhoid I, isolated from the first outbreak, is related but not identical with two strains of enteritidis, while Mouse Typhoid II is related to but not identical with the human paratyphoid B strains.

In a separate paper in this series, Webster[5] has identified Mouse Typhoid II strain with *Bacillus pestis caviæ* Smith and has suggested its close relation to the *Bacillus aertrycke* (mutton) group of Schütze.

[5] Webster, L. T., *J. Exp. Med.*, 1922, xxxvi, 97.

SURFACE TENSION OF SERUM.

III. Recovery after Lowering by Surface-Active Substances.

By P. LECOMTE du NOÜY, Sc.D.

(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

Plate 2.

(Received for publication, February 16, 1922.)

I.

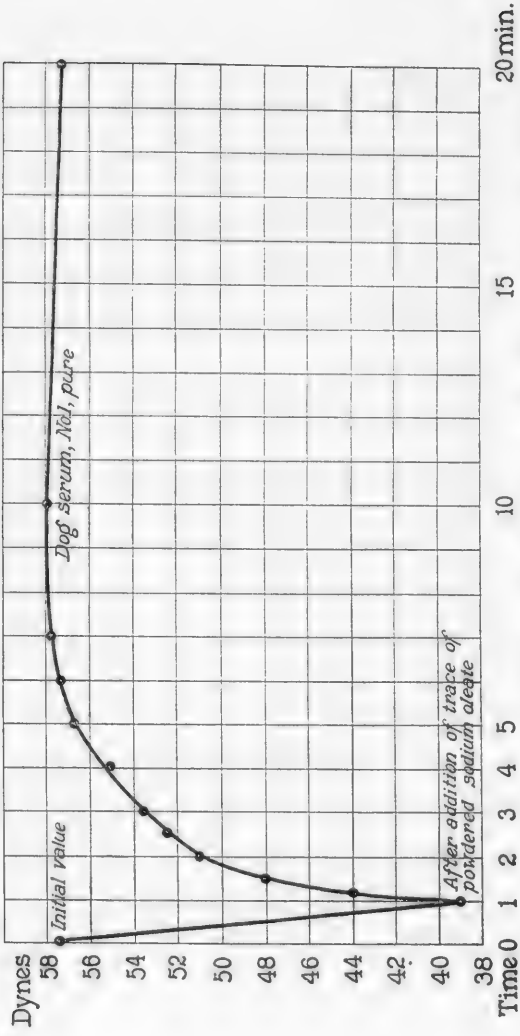
INTRODUCTION.

It has been shown in previous papers[1,2] that surface tension of serum and of solutions of serum varies in function of the time, and that the lowering of the surface tension seems to follow the laws of adsorption in the surface layer. The assumption was made that some of the constituents of the serum having a strong action upon the surface tension of water were actually adsorbed in function of time, or that certain changes in the arrangements of the group molecules took place in the surface layer.

Comparative experiments made with certain surface-active organic compounds, such as sodium oleate, glycocholate, and saponin, showed similar results.[2] But when sodium oleate, for instance, was added to serum or to a solution of serum, a converse phenomenon happened. A very considerable drop occurred first, immediately followed by a rise, which was function of the dilution of the serum and, in the case of pure serum, raised the surface tension to its normal value in a few minutes (Text-fig. 1). In other words, the action of sodium oleate was immediately counteracted and, in certain cases, entirely inhibited by an antagonistic or buffing action due to certain constituents of the serum. This explains why in jaundice large quantities of sodium

[1] du Noüy, P. L., *J. Exp. Med.*, 1922, xxxv, 575.

[2] du Noüy, P. L., *J. Exp. Med.*, 1922, xxxv, 707.



TEXT-FIG. 1. Rise of surface tension of pure serum after a drop due to the addition of sodium oleate.

glycocholate and taurocholate are present in the blood without producing any hemolysis. The same amount dissolved in saline solution would bring forth a strong lowering of surface tension and would hemolyze red cells powerfully. The purpose of this paper is the study of this phenomenon.

II.

EXPERIMENTAL.

The technique used was described in detail in the previous papers.[1,2] It will suffice to say that the measurements of the surface tension of the same layer of liquid were made by means of the du Noüy tensiom-

TABLE I.

Rise of Surface Tension of Serum in Function of Time after a Drop Due to the Addition of Sodium Oleate.

Pure Dog Serum, No. 1 (Text-Fig. 1).

Temperature 22°C.

About 1/10,000 in weight of powdered sodium oleate was used.

Time.	Surface tension.
	dynes
Before addition of sodium oleate	57.5
After " " " "	39.0
" 15 sec.	44.0
" 30 "	48.0
" 1 min.	51.0
" 1.5 "	52.5
" 2 "	53.5
" 3 "	55.0
" 4 "	56.8
" 5 "	57.3
" 6 "	57.6
" 9 "	58.0
" 20 "	57.6

eter. As usual, the utmost precautions were taken as to the cleanness of the glassware (watch-glasses, test-tubes, pipettes, stirring rods), which was washed in soda solution, then boiled for 30 minutes in a sulfuric acid and sodium dichromate cleaning fluid. Fresh dog and rabbit sera were used. When no graduated pipettes were needed, the

pipettes were chosen with very nearly the same capacity, measured by pressing evenly on the rubber nipple (1.8 to 1.9 cc.).

Immediately after the first measurement, a certain amount of sodium oleate was added and the surface tension measured. From 30 to 60 seconds elapsed between the two measurements. Although sodium glycocholate and taurocholate act in the same way, sodium oleate was chosen on account of its more marked action on surface tension. These measurements were made at regular intervals, generally 1 minute, at the beginning; in certain cases of very rapid recovery (pure serum), they were made at intervals of 15 seconds

TABLE II.

Rise of Surface Tension of Serum in Function of Time after a Drop Due to the Addition of Sodium Oleate.

Pure Dog Serum, No. 2 (Text-Fig. 2).

Temperature 22°C.

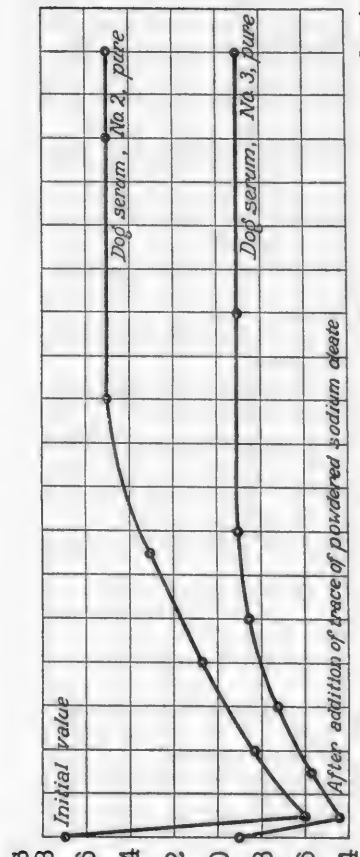
About 1/10,000 in weight of powdered sodium oleate was used.

Time.	Surface tension.
	dynes
Before addition of sodium oleate	56.0
30 sec. after addition of sodium oleate	46.0
After 1 min.	53.0
" 1.5 "	55.0
" 2.5 "	55.0

during the first 1 or 2 minutes. In order to avoid experimental errors, all the measurements were repeated, in certain cases four times.

1. Pure Serum.—The results obtained with Serum 1 are shown in Table I. This serum had previously been heated at 56°C. for 2 hours and did not manifest any sign of spontaneous lowering of surface tension when exposed in a watch-glass. After 9 minutes the value of the surface tension was higher than the initial value. An attempt to explain this fact will be made in a later paragraph. With Serum 2 (Table II and Text-fig. 2) the final surface tension was 1 dyne lower than the initial value.

With Sera 1, 2, and 3 (Tables I to III), the rise was extremely rapid, and after a drop of 18.5 dynes, the initial value was reached in 5



TEXT-FIG. 2. Rise of surface tension of pure serum after a drop due to the addition of sodium oleate.

minutes in the first experiment; in the second, the drop being much smaller (10 dynes), the initial value was reached in 1½ minutes, and in the third case (4.5 dynes), in 80 seconds. The same amount of sodium oleate added to saline solution brought its surface tension down to about 32.5 dynes permanently. Every serum seemed to react in its own specific way as far as time and final value were concerned.

The result obtained by the addition of 1 drop of sodium oleate solution at a concentration of 1/100 is given in Table IV and Text-fig. 3. The final concentration of sodium oleate in the solution was about 1/12,000. The concentration of sodium oleate was much smaller and

TABLE III.

Rise of Surface Tension of Serum in Function of Time after a Drop Due to the Addition of Sodium Oleate.

Pure Dog Serum, No. 3 (Text-Fig. 2).

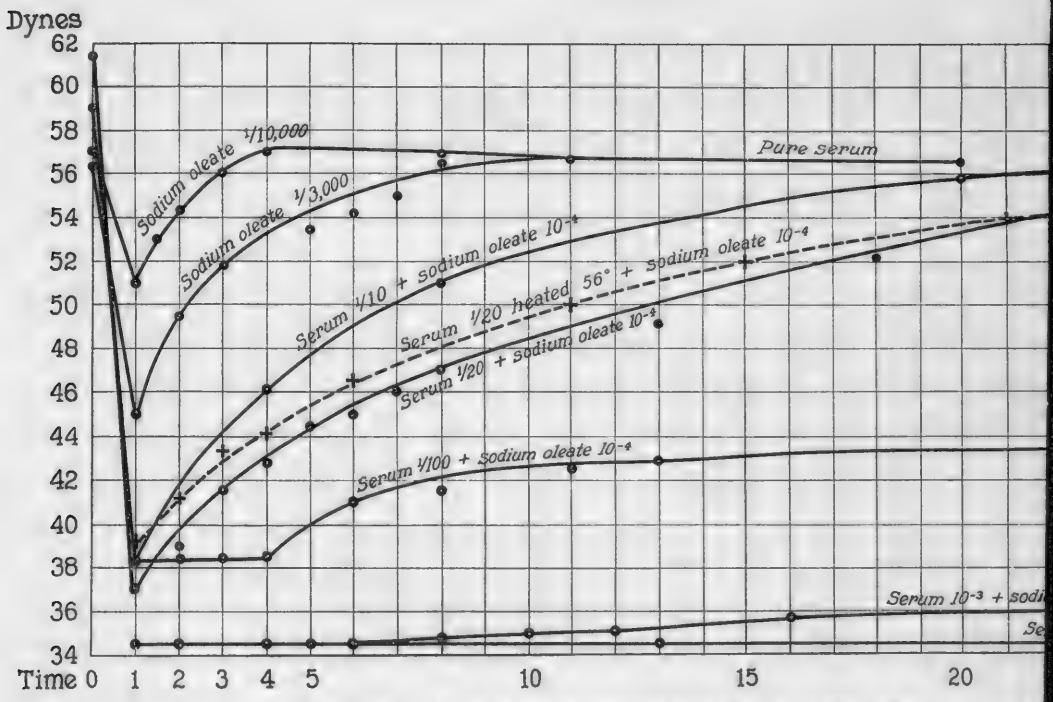
Temperature 22°C.

About 1/10,000 in weight of powdered sodium oleate was used.

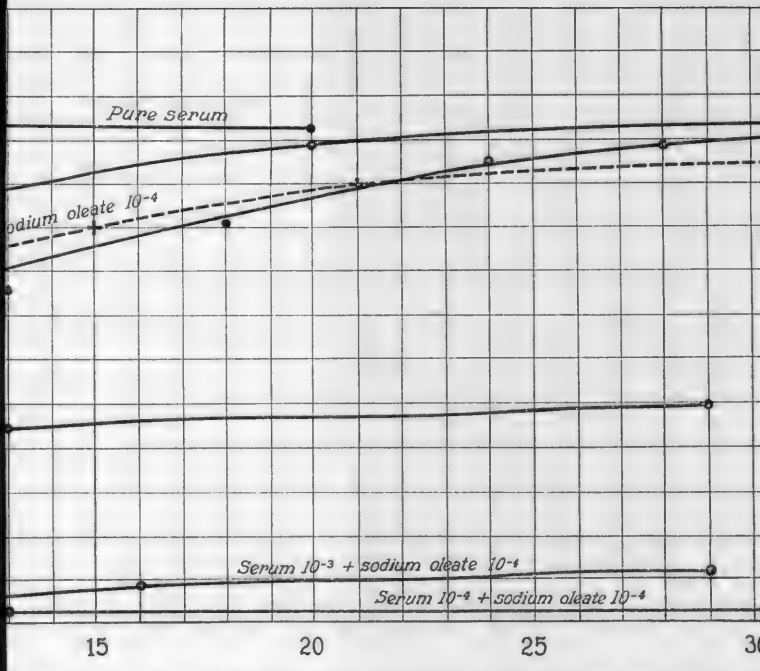
Time.	Surface tension.
	dynes
Before addition of sodium oleate	49.0
30 sec. after addition of sodium oleate	44.5
After 40 sec.	47.2
" 1 min.	48.5
" 1.3 "	49.0

the drop in surface tension was reduced accordingly. In Table V the same experiment was made, but 3 drops of the same sodium oleate solution were added instead of 1, making the concentration of the surface-active substance about 1/4,000. The final surface tension is equal to the initial value. It sometimes happens that after reaching its initial value rapidly, a drop occurs, due to the normal phenomenon studied in the preceding papers.[1,2]

2. Dilutions of Serum.—Dilutions of serum in saline solution at 0.9 per cent were prepared at concentrations of 1/10, 1/20, 1/100, 1/1,000, and 1/10,000. The same amount of sodium oleate was added to the same amount of solution. For these measurements, a 2 cc. graduated pipette was used. The average weight of a drop



TEXT-FIG. 3. Rise of surface tension of pure serum and serum solutions after a c



f pure serum and serum solutions after a drop due to the addition of sodium oleate

35 min.

was 0.019 gm. The amount of serum solution was equal to 1.9 gm. The final concentration of sodium oleate equalled 1/10,000 (Table VI).

TABLE IV.

Rise of Surface Tension of Serum in Function of Time after a Drop Due to the Addition of Sodium Oleate.

Pure Rabbit Serum, No. 119 (Text-Fig. 3).

Temperature 22°C.

1 drop of sodium oleate solution, 1/100, was used.

Time.	Surface tension.
	dynes
Before addition of sodium oleate	57.0
After " " " "	51.0
" 1 min.	54.3
" 2 "	56.0
" 3 "	57.0

TABLE V.

Rise of Surface Tension of Serum in Function of Time after a Drop Due to the Addition of Sodium Oleate.

Pure Rabbit Serum, No. 119 (Text-Fig. 3).

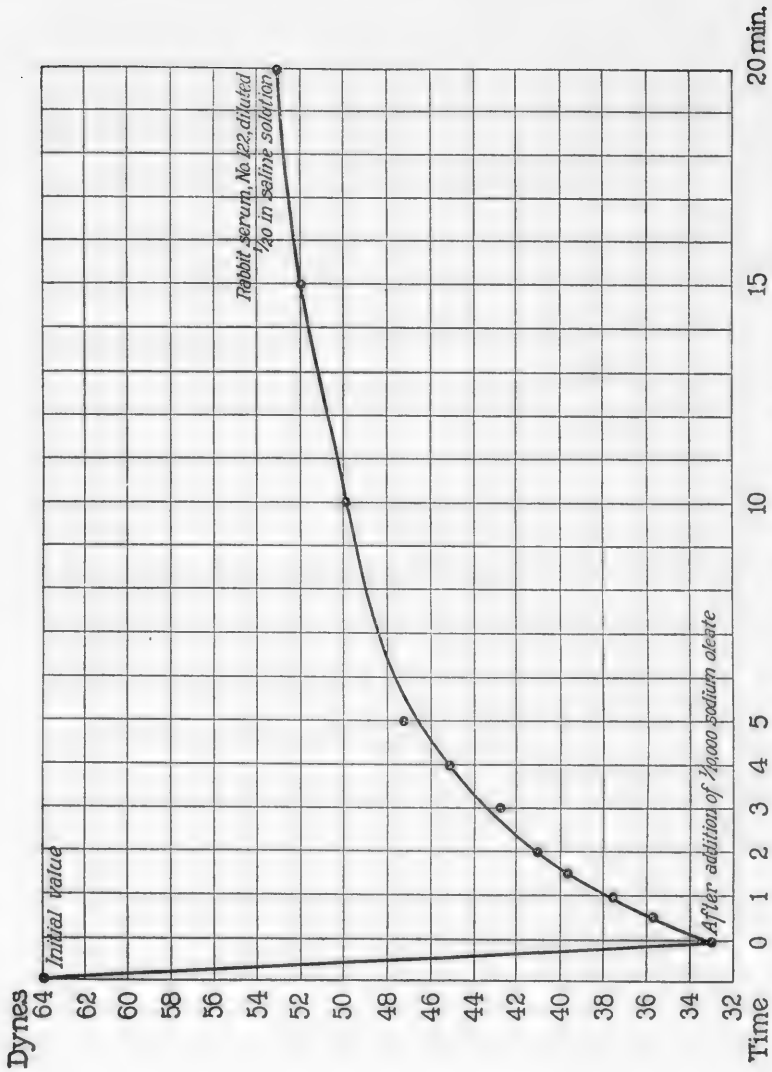
Temperature 22°C.

3 drops of sodium oleate solution, 1/100, were used.

Time.	Surface tension.
	dynes
Before addition of sodium oleate	56.3
After " " " "	45.4
" 1 min.	49.5
" 2 "	51.8
" 4 "	53.5
" 5 "	54.2
" 6 "	55.0
" 7 "	56.5
" 27 "	56.3

The recovery of a serum solution at 1/20 (rabbit serum, No. 122) is given also in Text-fig. 4.

These figures show that at a dilution of 1/10,000 the antagonistic power of the serum was inhibited. At the end of 24 hours, the



TEXT-FIG. 4. Rise of surface tension of a 1/20 serum solution after the addition of 1/10,000 of sodium oleate.

reading was still 35.6. This phenomenon is therefore quite different from that of the decrease in surface tension of serum solutions, which showed a maximum activity in most cases at a dilution of about 1/10,000.

The results in percentage rise and in absolute rise in 30 minutes are expressed by Text-figs. 5 and 6. For the same dilutions, the time

TABLE VI.

Rise of Surface Tension of Serum Solutions in Function of Time after a Drop Due to the Addition of Sodium Oleate.

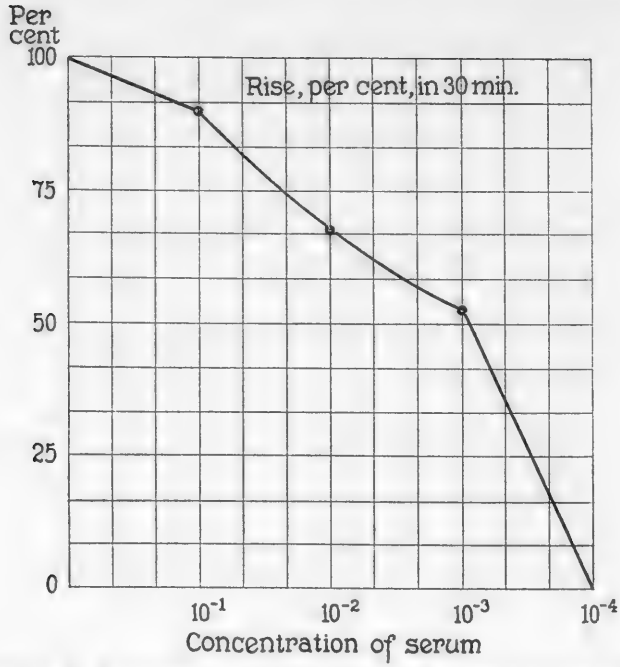
Rabbit Serum, No. 121, Diluted in Isotonic NaCl Solution (Text-Fig. 3).

Temperature 22°C.

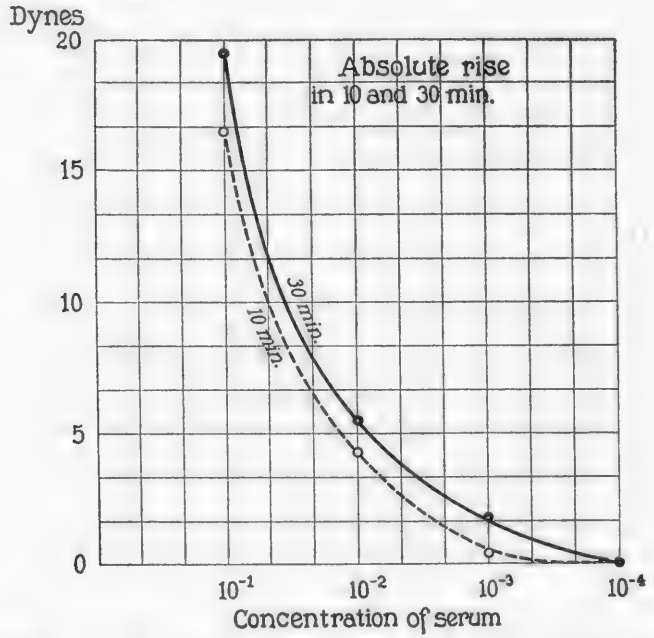
Sodium oleate, 10^{-4}, was used.

Time.	Surface tension. Concentration of serum.				
	1/10	1/20	1/100	1/1,000	1/10,000
	dynes	*dynes*	*dynes*	*dynes*	*dynes*
Before addition of sodium oleate.....	62.5	62.0	64.0	69.5	72.0
After " " " "	38.5	37.0	38.4	34.5	35.6
" 1 min.....		39.0	38.5	34.5	35.6
" 2 "	46.0	41.5	38.5	34.5	35.6
" 3 "		42.8		34.5	35.6
" 4 "	48.5	44.4	40.0	34.6	35.6
" 5 "		45.0	41.0	34.7	
" 6 "	51.0	46.0	41.7	34.8	
" 7 "		47.0			35.6
" 8 "	55.9			35.0	
" 9 "			42.6	35.2	
" 15 "	55.0	51.0	43.3	35.7	35.6
" 30 "	56.5	56.0	44.0	36.5	35.6

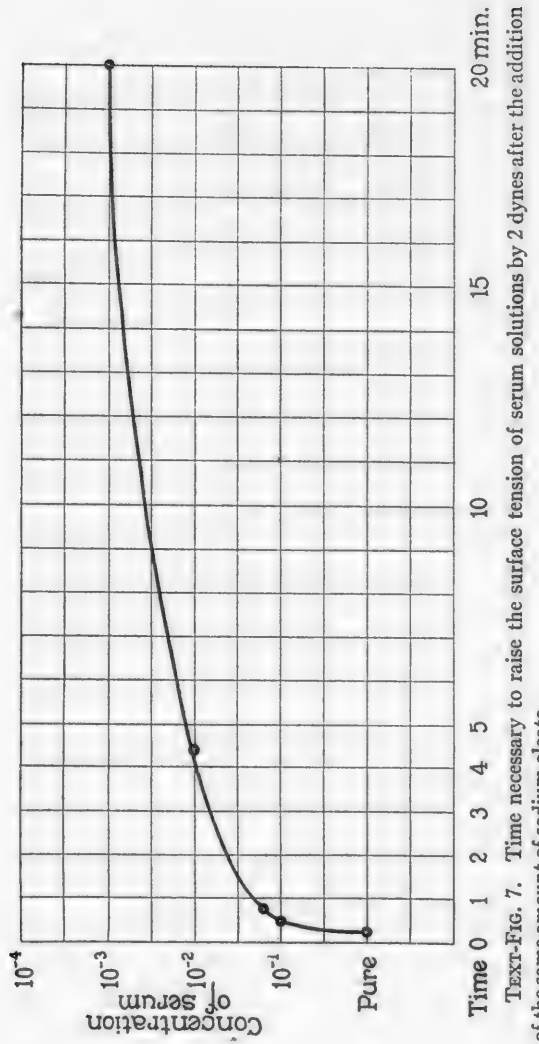
necessary to raise the surface tension of the solutions by 2 dynes is represented by Text-fig. 7. It will be seen that, by diluting the serum tenfold, the time is only doubled, while it is increased seventeenfold by diluting the serum solution again ten times; that is, to 1/100. Doubling the dilution at a concentration of 1/10, *viz.* 1/20, has the same effect as diluting pure serum tenfold. These phenomena, although difficult to interpret at present, are very probably due to the fact that surface tension is governed by the best possible arrange-



TEXT-FIG. 5. Percentage rise of surface tension of serum solutions and serum in function of the concentration of the serum after the addition of the same amount of sodium oleate. Time 30 minutes.



TEXT-FIG. 6. Rise of surface tension of serum solutions in function of the concentration of serum after the addition of the same amount of sodium oleate. Time 30 minutes.



TEXT-FIG. 7. Time necessary to raise the surface tension of serum solutions by 2 dynes after the addition of the same amount of sodium oleate.

ment of group molecules in the surface layer, as was stated in previous papers. The explanation of the broken aspect of the curve in Text-fig. 5 lies in the better knowledge of the structure of the

TABLE VII.

Quantitative Determination of the Antagonistic Action in Function of Time of a 1/10 Serum Solution.

Rabbit Serum, No. 120 (Text-Fig. 8).

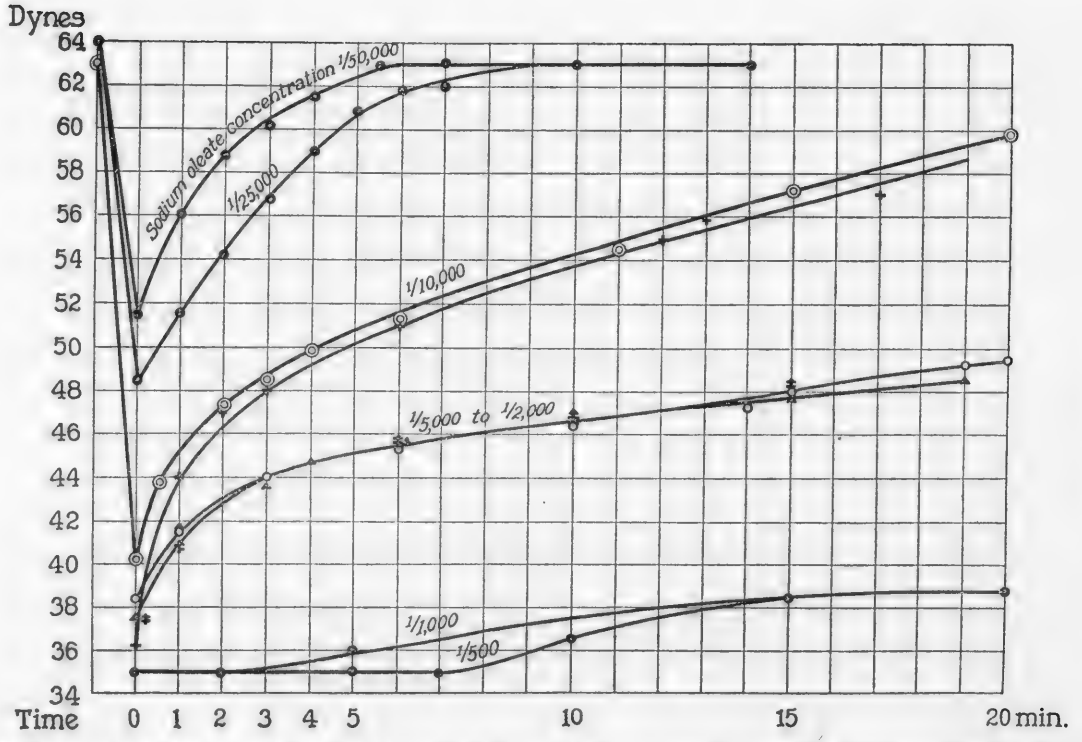
Temperature 22°C.

In every case, different amounts of sodium oleate were added to 1.9 gm. of serum solution. Each drop of sodium oleate weighed 0.019 gm.; 1, 2, 3, or more drops were added. Isotonic saline solution was used for the dilutions.

Time.	Surface tension. Concentration of sodium oleate. 1/50,000	1/25,000	1/10,000	1/5,000	1/2,500	1/2,000	1/1,000	1/500
	dynes	*dynes*	*dynes*	*dynes*	*dynes*	*dynes*	*dynes*	*dynes*
Before addition of sodium oleate.....	63.0	63.0	62.5	62.6	62.5	61.2	63.0	63.0
After " " " "	51.5	48.5	40.2	38.4	36.5	36.5	34.3	34.2
" 1 min.....	56.0	51.6	46.5	41.5	40.9	41.5	34.3	34.5
" 2 "	58.5	54.2	47.3	43.0	42.9	42.9	34.5	34.5
" 3 "	60.2	57.0	48.5	44.0	44.0	43.7	34.5	34.5
" 4 "	61.5	59.0	49.8	44.5		44.6	34.0	
" 5 "	63.0	60.8	50.5	44.7	45.0	45.0	36.0	
" 6 "	63.2	61.8	51.3		45.4	45.4		
" 7 "						45.8		
" 8 "		62.2						
" 10 "	61.0	63.0		46.4	46.6	46.6		
" 11 "			54.5					
" 13 "								38.6
" 15 "		61.0		48.0	48.0	47.5	38.4	38.6
" 16 "			57.9					
" 21 "			57.9					
" 30 "			61.0	51.6				
" 1 hr., 30 min.....			58.0	52.3			39.5	39.2

protein and other colloidal substances of the serum, and of their physical behavior when placed in contact with other free molecules.

In order to study the process of recovery of surface tension of serum solutions quantitatively, varying amounts of sodium oleate in solution were added to the same quantity of serum solution at 1/10 dilution. The results are given in Table VII and Text-fig. 8.



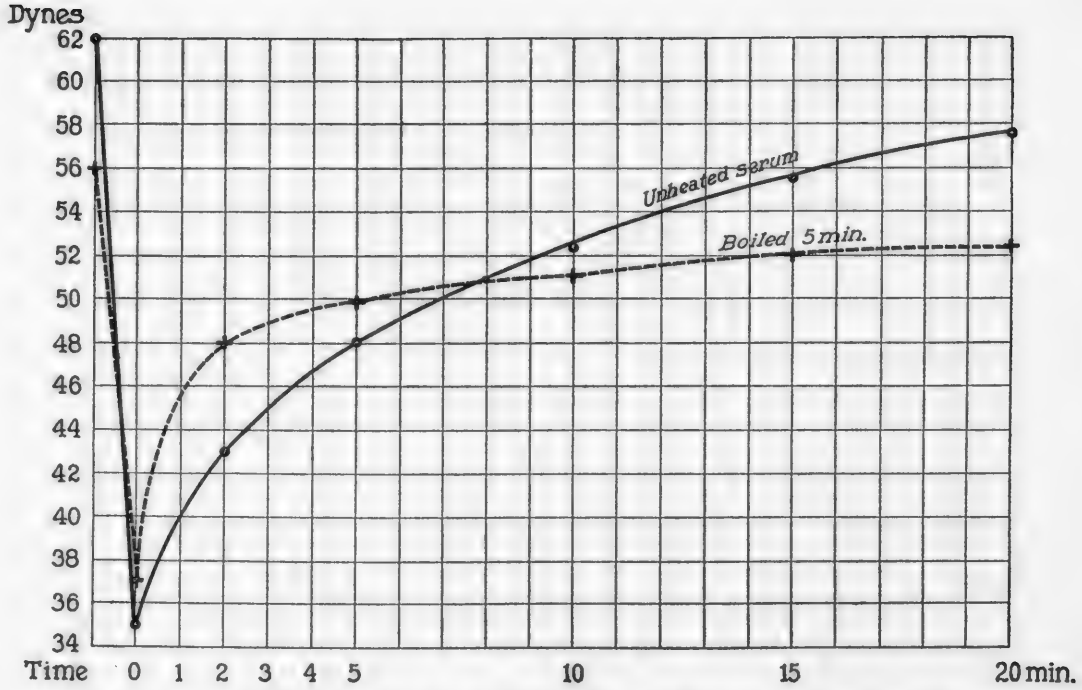
TEXT-FIG. 8. Rise of surface tension of a 1/10 serum solution after the addition of different amounts of sodium oleate.

It is decidedly important whether the solution is stirred or not after the addition of sodium oleate, or whether the surface-active substance is added powdered or in a concentrated or dilute solution. In other words, the results will be different if the concentration of 1/1,000 of sodium oleate is obtained by adding 1 drop of a 1/10, or 10 drops of a 1/100 solution of sodium oleate to the serum, compensation being made for the different amount of liquid. Generally speaking, the antagonistic action of the serum is more powerful when the sodium oleate is less dilute. It is strongest for pure powdered sodium oleate.

3. Action of Heat.—Heat modifies the properties of serum profoundly, even at a relatively low temperature such as 56°C. However, although the changes are important as far as the biological properties are concerned, they are undetectable to the chemist and the physicist. Heated serum is no longer the same, but were it not for biological tests, such as the study of hemolytic power, it would be impossible to differentiate it from unheated serum. Experiments on the recovery of the surface tension of serum solutions show that generally when a serum has been heated, the beginning of the recovery is more rapid, but that after 15 or 20 minutes, sometimes less, its curve crosses that of the recovery of the unheated sample, and remains below it. The serum acts practically in the same way whether heated before or after dilution. When heated before dilution, the phenomenon is slightly more marked. It was already known that dilution had a protective action on serum. The higher the temperature, the more marked the phenomenon, whether the serum was diluted in pure water or in saline solution. An idea of this phenomenon is given in Text-fig. 9.

In order to compare the sensitivities of the physiological and of the physical method, two samples of a 1/10 serum solution in saline solution were studied from the standpoint of the lowering of surface tension after the addition of sodium oleate. One sample was boiled in a water bath for 5 minutes, the other was not. Clark, Zinck, and Evans[3] observed that, after boiling, the protective action of human serum against hemolysis of red cells (guinea pig) by sodium oleate

[3] Clark, H. M., Zinck, R. H., and Evans, F. A., *Bull. Johns Hopkins Hosp.*, 1921, xxxii, 328.



TEXT-FIG. 9. Rise of surface tension of a 1/10 serum solution before and after heating 5 minutes at 100°C.

was decreased. In other words, it required a smaller concentration of sodium oleate to produce the same degree of hemolysis (1/50,000 with fresh serum, 1/65,000 with boiled serum). We were unable to check up these results by using rabbit serum and hen cells, but our measurements showed that the surface tension of the boiled serum solution was inferior by 10 dynes to that of fresh serum, which would suffice in itself to explain a difference, and that the recovery, although more rapid in the first 8 minutes, was much below that of the fresh serum at the end (Text-fig. 9.) When using serum solution in distilled water (dilution 1/20), the action was similar (Text-fig. 10).

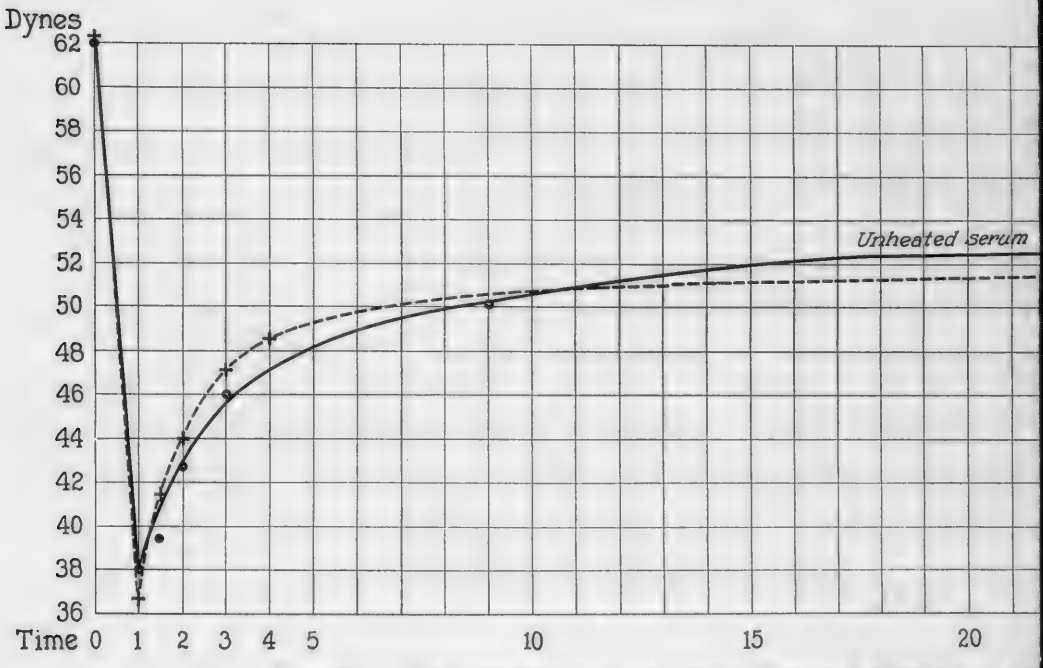
In this case, the study of the surface tension shows that the phenomenon is dependent on two factors; the initial value of the surface tension plays one part, and the antagonistic action of the serum another. The advantage of the physical method was that it decomposed a complex phenomenon into its elements, introduced the factor time, and did not depend on such an unreliable standard as hemolysis of red cells.

The considerable modifications brought about in the serum by temperature are also very strikingly illustrated by Figs. 1 to 4. These are photographs of the crystallization of four samples of a 1/10 solution of serum in saline solution, to which 1/10,000 of sodium oleate had been added. The first sample (Fig. 1) was unheated. The second (Fig. 2) was heated at 56°C. for 2 hours. The third (Fig. 3) was heated at 70°C. for 1 hour, and the fourth (Fig. 4) was boiled for 5 minutes. These phenomena will be studied in another paper.

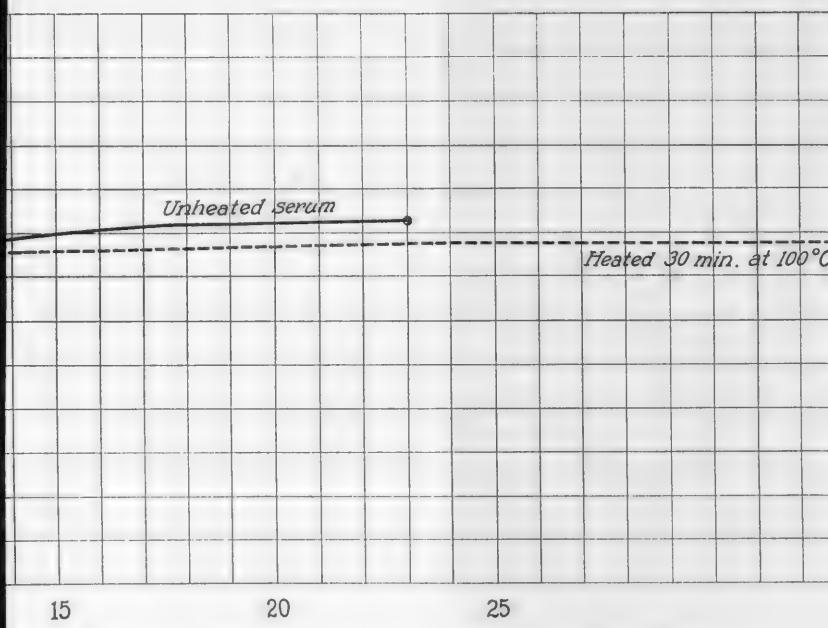
III.

DISCUSSION.

All these facts may be accounted for by simply assuming that this phenomenon of recovery is not due to a special substance but to the adsorption of the surface-active molecules by the large colloidal micellæ of the serum. Comparison of the curves of recovery with ordinary curves of adsorption makes this plain, but an attempt was made to obtain the same phenomenon with inert colloidal solutions, such as gelatin and gum arabic. If our hypothesis



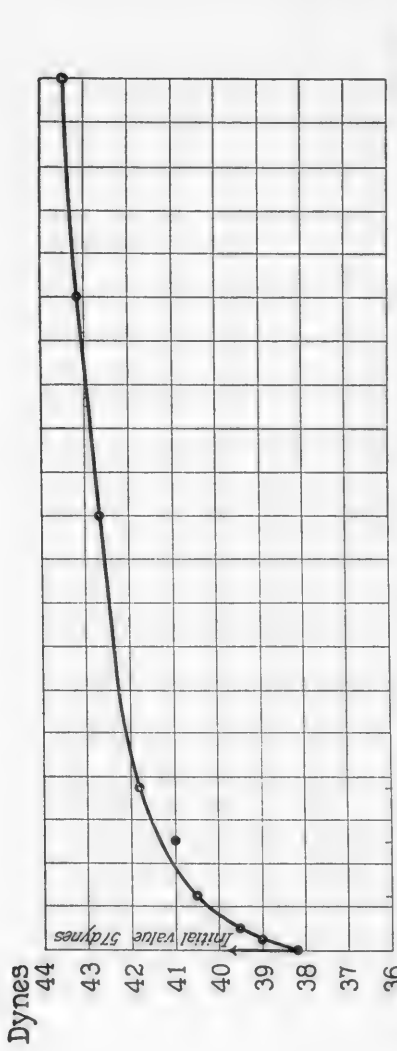
TEXT-FIG. 10. Rise of surface tension of a 1/20 serum solution in distilled water befo



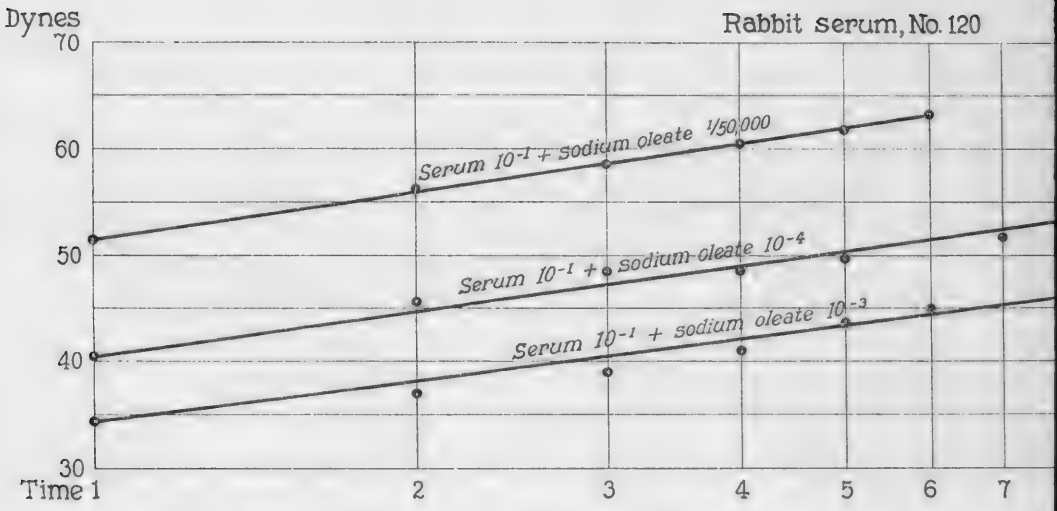
0 serum solution in distilled water before and after heating 30 minutes at 100°C.

35 min.

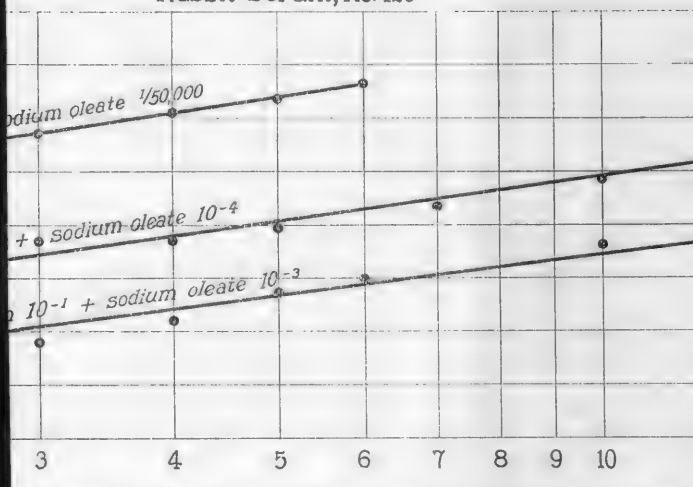
were correct, the same recovery should take place as soon as *any* colloidal particles should be present in an aqueous solution whose surface tension had been lowered by the addition of sodium oleate. The order of magnitude of the phenomenon depending mainly on the size of the particles, a much slower process was expected. Experiments showed the correctness of the views expressed above (Text-fig. 11), solutions of gelatin, egg albumin, and gum arabic acting in a way absolutely comparable to that of serum. When sodium oleate, powdered or in highly concentrated solution, is added to the serum, without much stirring, the oleate spreads all over the free surface, forming large aggregates of molecules which are rapidly adsorbed by the serum micellæ of the surface layer, some of them becoming heavy enough to fall to the bottom. At a certain optimum concentration, almost all the oleate molecules are being adsorbed; that is, withdrawn from the surface. They form a thick coating around the molecules or group molecules of serum, which tends to force them apart a little more than they were before. This explains why the surface tension, after recovery, often reaches a slightly higher value than before the addition of oleate. However, a great number of micellæ in the bulk are free from sodium oleate molecules and they become progressively adsorbed in the surface layer, thus lowering slightly the surface tension, while the sodium oleate-coated micellæ remain in the bulk or are precipitated, and the surface tension falls back to normal. On the contrary, should a dilute solution be used, the oleate molecules are very mobile. No large aggregates are formed, the solution mixes readily with the serum, the surface-active molecules are brought near every serum molecule in the bulk, adsorption follows, but the result is that *all* serum micellæ carry a certain amount of oleate molecules so that the recovery is slow and the surface tension may never reach as high a value as in the case in which concentrated oleate solution was used. Of course, in the case of powdered or highly concentrated oleate, stirring will produce the same effect as if the solution were more diluted. But when the solution is stirred only slightly or not at all, it is easy to observe the occurrence of a visible and rather coarse precipitate, while if the solution has been vigorously stirred, it remains clear and merely opalescent. When a solution of 1/10 of



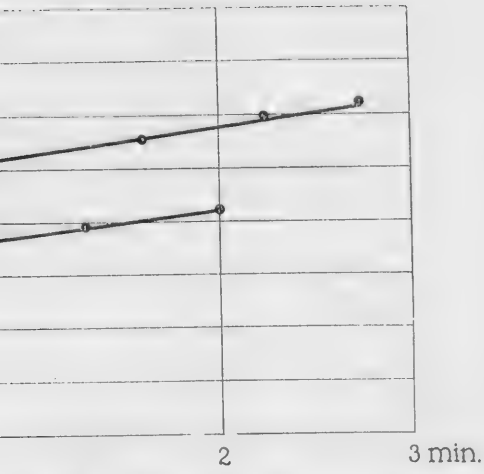
TEXT-FIG. 11. Rise of surface tension of a 1/50 solution of gum arabic in saline solution 0.9 per cent after the addition of 1/30,000 of sodium oleate.



TEXT-FIG. 12. Rise of surface tension of a 1/10 serum solution after the addition of different amounts of s



solution after the addition of different amounts of sodium oleate (curves o



Text-fig. 8), plotted on semilogarithmic paper.

oleate is used, films are seen floating at the surface. This never occurs with a solution of 1/100.

In our experiments, the phenomenon of recovery was not a simple function of the concentration of sodium oleate; whether the concentration of oleate was 1/5,000 or 1/2,000, the rate of recovery was almost identical at the beginning, while there was a considerable difference between the rates of recovery of solutions at 1/10,000 and 1/5,000 (Text-fig. 8). The same gap exists between 1/2,000 and 1/1,000 while 1/1,000 and 1/500 act practically in the same way. Too little is known about the structure of the serum molecules and the intimate mechanism of the surface tension to attempt a quantitative explanation of these facts. There is no doubt but that the solution of the problem will be found in the knowledge of the interactions of the group molecules in the surface layer. The curves (Text-fig. 8) correspond to possible geometrical arrangements of the group molecules, resulting in certain optima stray fields. These facts will only be cleared up some day by a thorough study of the physics of the molecules, probably on the basis of Langmuir's theory.[4]

Some of the curves of recovery of surface tension were plotted on semilogarithmic paper and appear as straight lines (Text-fig. 12). This indicates that the process follows the compound interest law, and may be expressed by an equation of the form

$$\gamma = ae^{bx}$$

similar to that established for the phenomenon of spontaneous lowering of surface tension,[1,2] and adsorption in general.

IV.

CONCLUSIONS.

1. The equilibrium of the serum corresponding to its normal minimal surface tension is as stable and difficult to break, under ordinary conditions, as the osmotic tension equilibrium. The addition of a strong surface-active substance (sodium oleate, glycocholate, or taurocholate), will not lower it definitely, unless the substance is present in large amounts and in solution. After the first rapid drop

[4] Langmuir, I., *J. Am. Chem. Soc.*, 1917, xxxix, 1848.

has occurred, a process of recovery takes place, which brings back the normal surface tension in a short time (from 2 to 6 minutes in the case of pure serum). As a drop in the surface tension of the serum of animals may be very injurious to the red cells, this process of recovery is a normal one of defense in all cases in which surface-active substances (bile) are set free in the blood.

2. When diluted, the serum shows the same phenomenon to a smaller extent; the time of recovery is very much longer and the final surface tension is always lower than the original value. At a dilution of 1/10,000, no recovery takes place, the dilution being too high to overcome the lowering action of 1/10,000 of sodium oleate.

3. The recovery is stronger when the surface-active substance is added powdered or in a highly concentrated solution, and not stirred.

4. The recovery does not seem to be inversely proportional to the concentration of sodium oleate, when added superficially. Doubling the concentration at 1/2,000, for example, gives the same curve of recovery. This happens under certain conditions; namely, when the liquid is not stirred after the addition of sodium oleate.

5. This recovery is due to a purely physical phenomenon, namely adsorption, and is not specific for the serum. Other colloidal solutions, such as gum arabic, egg albumin, gelatin, and silver and gold sols, show it, only to a smaller degree. The process of recovery follows a logarithmic law in all cases, expressed by an equation of the form

$$\gamma = ae^{bx}$$

6. Temperature affects this phenomenon. At first it enhances it, but finally decreases it. This would seem to connect the loss of the property of the serum known as complement in a serum with a modification of the physical properties of this serum. This phenomenon is being investigated further.

EXPLANATION OF PLATE 2.

FIGS. 1 to 4. Action of temperature on the crystallization of serum solutions, concentration 1/10 in saline solution 0.9 per cent (rabbit serum).

FIG. 1. Unheated.

FIG. 2. Heated at 56°C. for 2 hours.

FIG. 3. Heated at 70°C. for 1 hour.

FIG. 4. Heated at 100°C. for 5 minutes.

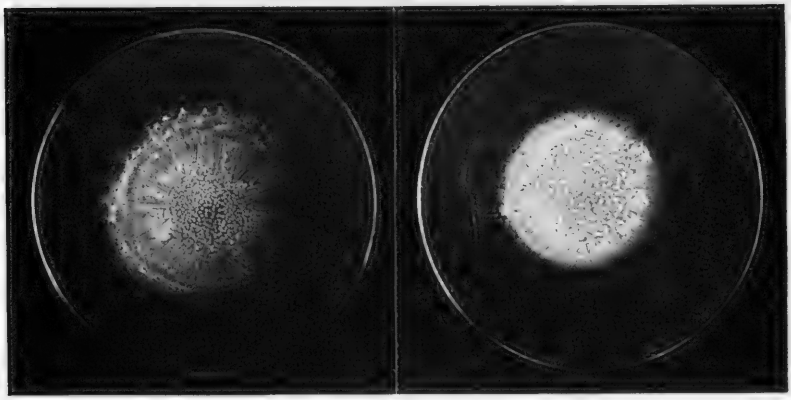


FIG. 1. FIG. 2.

FIG. 3. FIG. 4.

(du Noüy: Surface tension of serum. III.)

INFECTIOUS MOTOR PARALYSIS IN YOUNG RABBITS.*

By J. HOMER WRIGHT, M.D.,† and EUGENE M. CRAIGHEAD.‡

(From the Pathological Laboratory of the Massachusetts General Hospital, Boston, Mass.)

PLATES 3 AND 4.

(Received for publication, February 18, 1922.)

The paralysis described in this article was observed during experiments planned to confirm, if possible, the rat and flea transmission theory of infantile paralysis as outlined by Richardson.[1,2] The investigations began about February 1, 1919, and continued over a 10 month period. In general it was planned to infect young rabbits through intracerebral inoculations with the virus of infantile paralysis and then to determine whether the infection could be transferred from the sick animal to the healthy through the bites of fleas.

For the primary intracerebral inoculations four viruses were secured.[3] The rat flea (*Ceratophyllus fasciatus* Bosc[4]) was used in most of the experiments. In a few cases dog and cat fleas (*Ctenocephalus*

* This work has received financial support from a fund donated by the following gentlemen: James J. Storrow, Charles Jackson, Wallace Pierce, Arthur Perry, James H. Proctor, R. L. Studley, Allan Emery, William Endicott, Frank G. Webster, and Frank W. Hallowell, of Boston, Mass.; J. P. Morgan and Albert Strauss, of New York; Alvah Crocker and George R. Wallace, of Fitchburg, Mass.; and Winthrop Murray Crane, of Dalton, Mass.

† Pathologist, Massachusetts General Hospital, Boston, Mass.

‡ Scientific Assistant, Bureau of Plant Industry, Harrisburg, Pa.

[1] Richardson, M. W., The rat and infantile paralysis—a theory, *Boston Med. and Surg. J.*, 1916, clxxv, 397.

[2] Richardson, M. W., The rat and infantile paralysis—a theory, *Am. J. Pub. Health*, 1918, viii, 564.

[3] Two straight monkey viruses were kindly furnished by Dr. George W. McCoy of the United States Hygienic Laboratory, Washington, D. C., and two mixed monkey viruses by Dr. Simon Flexner of The Rockefeller Institute for Medical Research.

[4] Craighead, E. M., Observations on certain Siphonaptera, *Entomol. News*, 1921, xxxii, 303.

canis Curtis, and *Ctenocephalus felis* Bouche) were used. In the preliminary intracerebral inoculations from 1 to 0.5 cc. of a suspension of glycerolated spinal cord in $\frac{N}{1}$ salt solution was used.

Fifteen rabbits were inoculated intracerebrally with virus from the Hygienic Laboratory without pathological effect. Eight guinea pigs and three rabbits were inoculated intracerebrally with Rockefeller Institute virus, and all remained well but one rabbit, No. 12. Beginning 12 hours after inoculation fleas were allowed to feed alternately first on inoculated and then on normal animals, and this sequence was continued daily until the fleas died, or until the secondary animal showed paralysis. About 150 fleas were used at each feeding.

March 20, 1919. Rabbit 12 (5 weeks old) was inoculated intracerebrally with 0.1 cc. of a suspension in $\frac{N}{1}$ salt solution of glycerolated spinal cord. March 25. The animal showed some tremor and developed slight paralysis of the fore quarters. April 22. Died. The pathological findings were negative.

Fleas which had bitten Rabbit 12 were transferred daily to Rabbits 12 B and 18 (7 days old), and the latter developed typical paralysis dying 33 and 40 days respectively after their first flea bites. There was never any possible physical contact with Rabbit 12. Under similar conditions nine other rabbits became paralytic and died, the intervals between the first flea bites and death ranging from 9 to 40 days.

It seemed, therefore, highly probable that a paralytic disease having its origin in human infantile paralysis had been induced in young rabbits and transferred from animal to animal by the bites of fleas. The fallacy of this conclusion was quickly demonstrated, however, by the proper control experiment, in which three normal rabbits were simply placed in cages with the sick rabbits, the intermediate biting of fleas being omitted. Two of these control rabbits developed paralysis and one died.

Thus we had to do, presumably, with a paralytic disease in young rabbits spread by simple contact. Further investigation, moreover, showed it to be associated with a large organism which, as will be seen later, can only by the remotest possibility, have anything to do with human infantile paralysis.

Clinically this rabbit disease is characterized by a persistent drowsiness, followed in most cases by tremor shortly before the development of paralysis. The paralysis may be slight or marked, general or localized, and the mortality is high.

During the disease period stained blood smears were examined daily, but nothing suggestive of microorganisms was found. In ten cases cultures were made upon every available medium from brain, cord, kidney, spleen, and liver but no growth occurred. Cultures and smears from the digestive systems and salivary glands of fleas gave negative results. Attempts to transmit the disease through the bites of bedbugs and mites were negative. Intracerebral inoculations of sedimented urine, also of cord emulsions from sick rabbits, reproduced the paralytic disease in other rabbits.

Examination of the infected rabbits showed the following gross and microscopic factors and conditions.

Spinal Cord and Brain.—The cords from nine animals, together with the brains of five of them, were examined. No definite gross lesions were apparent except softening of the cord.

Microscopic sections show small focal lesions in both the gray and white matter, which rarely occupy an area as large as the field of an oil immersion lens (Fig. 1). They consist in infiltration by small inflammatory cells with rounded or irregular shaped nuclei. Polymorphonuclear leucocytes do not take part in the infiltration. The nerve cells in the infiltration areas are diminished in number or absent. Occasionally a degenerated or necrotic nerve cell is present. The foci of infiltration in the white matter are associated with capillary blood vessels.

The lesions are present in one or more sections of the cord, or cord and brain, of each of the nine animals. In a cross-section of a cord one or more lesions (up to about half a dozen) may be present. In a few instances there is mononuclear cell infiltration about the vessels of the pia mater.

By appropriate staining methods peculiar bodies are demonstrable in most of the lesions. These are considered to be microorganisms and will be referred to as such. They are elongated, sharply and smoothly outlined, with rounded or conical ends, and are slightly variable in shape and dimensions. Their length and thickness are

probably never more than 4 and 1.5 microns respectively. As a rule, they do not stain homogeneously, but show one or two lighter staining areas. In general appearance they closely resemble bacilli. They stain by Gram's method and with methylene blue. Wright's stain does not show any nuclear structure in them and stains them only faintly. They are acid-fast to a certain extent. The best method of demonstrating them consists in first staining the section with carbolfuchsin (diluted 1:4), mordanting and decolorizing with undiluted formaldehyde,[5] and counterstaining with methylene blue. By this method the microorganisms are stained red and the nuclei of the cells blue.

The microorganisms are found in varying numbers in the lesions. They occur scattered among the infiltrating cells and occasionally, in large number, in nerve cells, or in compact aggregations in smoothly outlined rounded or oval spaces in the tissue, of about the size of a nerve cell. The cytoplasm of a nerve cell containing the microorganisms may be vacuolated and the cell body more or less transformed into a shell (Fig. 2). The spaces filled with microorganisms seem to represent destroyed nerve cells. In one instance a nerve cell in a spinal ganglion is thus apparently represented by a compact mass of the microorganisms.

Nerve cells invaded by the microorganisms and the aggregations in spaces are not always closely associated with the focal infiltration by inflammatory cells, and there may be no evidence of inflammatory reaction about them. The appearances seem to indicate that invasion and destruction of nerve cells is the first step in the development of some of the focal lesions.

Kidney.—The kidneys from five rabbits all showed essentially the same lesions, which were either not apparent to the naked eye or appeared as grayish spots and streaks on the cut surface. The lesions are chiefly in the medulla. They consist essentially of multiple small foci of infiltration by small cells with round, indented, or fragmented nuclei, and are marked by degeneration and disappearance of the epithelial cells in the infiltrated areas. Microorganisms like

[5] The use of formaldehyde for this differential staining of the microorganisms was suggested by Dr. William H. Smith, who has long employed it successfully as a mordant in staining certain bacteria.

those in the central nervous system are present. They occur chiefly in closely packed aggregation in epithelial cells and in the lumina of tubules in varying numbers along with free cells and detritus (Fig. 3). In the denser infiltrated areas they are generally few or not demonstrable. The number of epithelial cells harboring the microorganism is relatively small and they are scattered. In sections stained with fuchsin and methylene blue, as above described, there may be seen in tubules, along with the characteristic red-staining microorganisms, blue-staining bodies of varying shape and size which seem to be degenerated forms.

The microorganisms were found in the urine during life. The staining method above described served to differentiate them from the bacilli present, which stained blue (Fig. 4).

Spleen.—Sections of the spleen from four rabbits show the presence in the pulp of large mononuclear phagocytic cells containing what seem to be degenerate forms of the microorganisms. These are variable in size and shape and show an affinity for methylene blue instead of fuchsin.

Liver.—A section from only one animal was examined and this showed two or three small focal lesions.

Myocardium.—Sections from two animals were examined and a few focal lesions found.

SUMMARY.

1. The attempt to infect young rabbits and guinea pigs with material containing in all probability the virus of human infantile paralysis failed.

2. Failure to infect the primary animals almost of necessity brought failure with the secondary flea-bitten animals. It is, however barely conceivable that a non-infectious form of an organism might circulate in the blood of the primary animal and that this form, through development in an intermediate host, the flea, might become virulent for the secondary flea-bitten animal.

3. Incidentally, and presumably accidentally, a paralytic disease was observed in young rabbits associated with the presence of an organism showing certain definite characters. So far as we know this paralysis and the associated organism have not been previously described.

4. This organism is found widely distributed in the organs of the affected animals and can be demonstrated in the urine. The active destruction by the organism of the nerve cells of the spinal cord is particularly striking, and gives complete explanation for the paralysis observed clinically.

5. With the organism present in the urine the spread of the disease by contact can be easily understood.

6. The transfer of the infection from animal to animal by fleabites is possible but not probable.

7. The nature of the observed organisms is in doubt. They represent probably an intermediate stage in the life history of some protozoan parasite.

EXPLANATION OF PLATES.

PLATE 3.

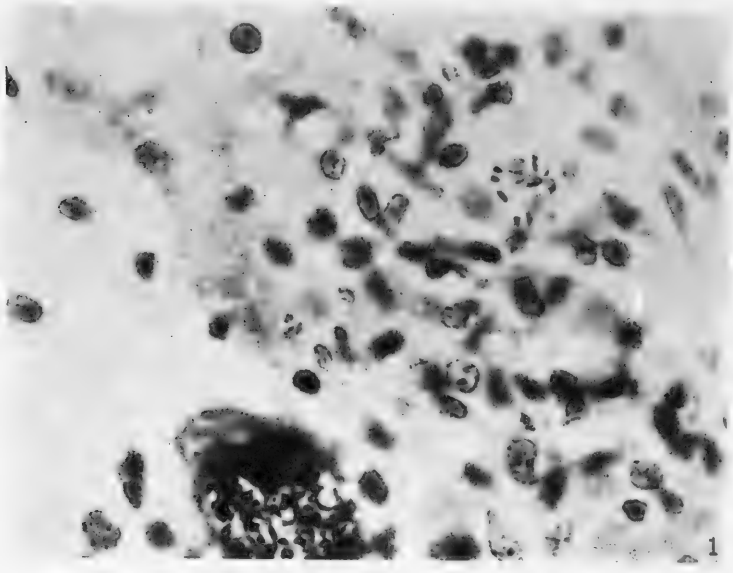
FIG. 1. A focal lesion in the spinal cord showing the infiltration by cells, and the microorganisms, many of which are in a degenerated nerve cell at the lower margin. × 1,000.

FIG. 2. Nerve cell of the spinal cord, degenerated and invaded by many of the microorganisms. A few microorganisms lie outside of the cell. × 1,000.

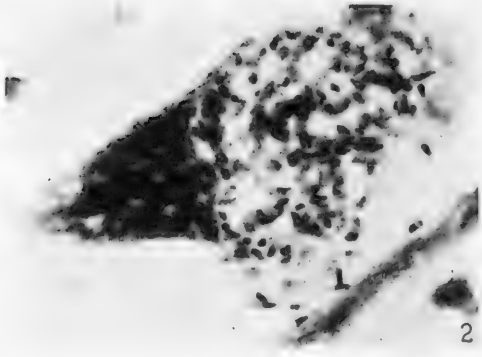
PLATE 4.

FIG. 3. Groups of microorganisms in four epithelial cells of the kidney. A sparse inflammatory cell infiltration is also shown. × 1,000.

FIG. 4. A mass of microorganisms in a smear preparation from a urinary sediment. Some bacilli are also present. × 1,000.

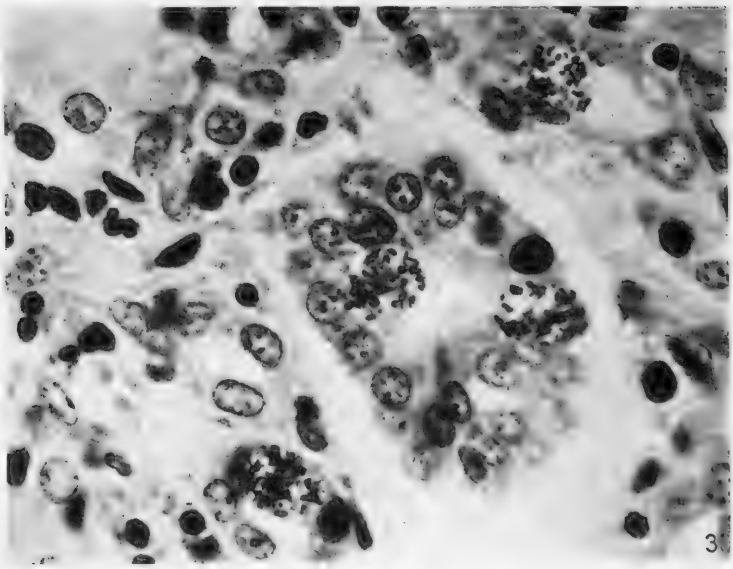


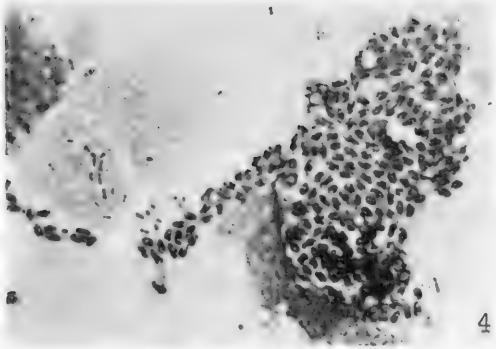
1



2

(Wright and Craighead: Infectious motor paralysis.)





(Wright and Craighead: Infectious motor paralysis.)

THE STRUCTURE AND DIFFERENTIATION OF THE SPECIFIC CELLULAR ELEMENTS OF THE PARS INTERMEDIA OF THE HYPOPHYSIS OF THE DOMESTIC PIG.*

By SIEGFRIED MAURER, M.D., and DEAN LEWIS, M.D.

(From the Hull Laboratory of Anatomy of the University of Chicago, and the Department of Surgery of Rush Medical College, Chicago.)

Plate 5.

(Received for publication, January 11, 1922.)

Since the discovery by Oliver and Schäfer that the hypophysis contains a specific substance which causes, when injected into the blood serum, a rise of blood pressure, much interest has been displayed in the question as to the source of this material and the mode of its discharge from the gland.

The observations of Howell, which have been amply confirmed, definitely established the origin of the pressor material exclusively from the posterior lobe of the gland, but left unsettled the question whether the active portion of this lobe is the epithelial investment derived from the pouch of Rathke, or the nervous portion derived from the brain.

Many efforts have been made to recognize histologically the antecedents of the secretion in the several parts of the posterior lobe. Of particular interest in this connection are the observations of Herring (1908), since they have given rise to a conception of posterior lobe secretion which has been supported by later experimenters, and widely adopted by those interested in the problems of internal secretion.

Herring studied in particular the relations of the pars intermedia, or epithelial investment, to the nervous substance of the posterior lobe. In the former he saw frequently vesicles wholly or partly formed of epithelial cells and filled with a material which resembled, under the microscope, the colloid of the thyroid vesicles. In the pars nervosa also numerous small masses of a hyaline material sometimes homogeneous, sometimes finely granular, were found. These hyaline masses were found throughout the substance of the pars nervosa and gave the impression of materials flowing towards the recess of the infundibulum, in the cavity of which also similar deposits were found. Herring expressed himself with considerable

* The expenses of this investigation were provided by a fund donated by Mrs. Nettie F. McCormick of Chicago, to whom the authors wish to express their grateful appreciation.

reserve concerning the nature and origin of these hyaline bodies but considered two possibilities. He noted that they were found frequently between the ependymal cells near the ventricular cavity. He considered this as suggestive of their being the product of ependymal cells, but remarked that the hyaline was not always confined to this situation since it was frequently found among the cells of the epithelial investment, a fact pointing strongly to its origin from the epithelial cells. In some cases the hyaline bodies were apparently surrounded by a layer of cells resembling endothelium, as if they were contained in lymphatic vessels.

In a later study of the changes in the hypophysis produced by complete thyroidectomy in rabbits and dogs, Herring found proliferation of the cells of the pars intermedia with invasion of the pars nervosa, and an enormous quantity of granular, hyaline, or colloid bodies in the body of the pars nervosa and in the laminæ forming the floor of the third ventricle of the brain. Concerning these changes he says: "The significance of these changes is as yet undetermined. The colloid bodies appear to arise from the epithelial cells of the pars intermedia, and their extensive production to be an exaggeration of a normal process."

Crowe, Cushing, and Homans (1910) in their study of the results of partial and complete hypophysectomies in the dog reached similar conclusions. Referring to Herring's observations they say: "and we agree with him that the histological appearances point strongly toward the fact that this is an actual secretion and one which appears to be the product of activity of the cells of the epithelial investment (pars intermedia). The material seems to pass toward the infundibulum and in many cases may actually be seen passing into the cavity of the third ventricle." According to these authors neither pure intermedia substance nor the colloid of the pars intermedia vesicles has any pressor effect. They suggest, therefore, that the pars nervosa activates the hyaline bodies "at least if they are in any way related to the glandular colloid" during their passage toward the infundibular cavity.

Cushing and Goetsch (1910–11) meanwhile have confirmed the results of Herring's observations as to the increase of hyaline bodies, etc., after thyroidectomy, and found a similar increase after a number of other experimental procedures; for example, after total pancreatectomy, mechanical injuries, partial hypophysectomies, and after experimental obstruction of the stalk by means of a silver clip applied about it. These authors accept unreservedly Herring's conception of a secretory product passing through the pars nervosa and believe that it is discharged into the cerebrospinal fluid. They also state definitely that they believe the colloid accumulation in newly formed epithelial vesicles is merely a precursor of the hyaline bodies, though the latter apparently may originate by a direct transformation of individual wandering epithelial cells. These authors also obtained with human cerebrospinal fluid concentrated by evaporation, injected intravenously in dogs and rabbits, unmistakable evidences of the presence in the fluid of a pressor substance similar to that obtained from extracts of the posterior lobe of the hypophysis.

Carlson and Martin (1911–12), on the other hand, failed to get any pressor response whatever from the intravenous injection of dog cerebrospinal fluid into dogs. It must be noted, however, that these workers did not concentrate the cerebrospinal fluid prior to testing it, as did Cushing and Goetsch. Carlson and Martin suggest that the pressor response obtained by Cushing and Goetsch may have been due to their using foreign, pathological, and concentrated cerebrospinal fluid.

The net result of these various researches is an expansion of Herring's views. The pars intermedia elaborates a specific secretion which it discharges into the pars nervosa either in the form of colloid or by the degeneration of pars intermedia cells which have migrated into the pars nervosa. The secretion appears in the pars nervosa as hyaline bodies either free in the interstices of the glia or inside of lymphatic channels. It finally reaches the ventricle and is discharged into the cerebrospinal fluid. This product contains the pressor substance of Oliver and Schäfer. It is to be noted, however, that Herring recognizes the alternative possibility that the hyaline bodies may be a product of the activity of ependymal cells.

In order to establish this hypothesis it is necessary to prove that the active substance of the secretion is present in the epithelial structure from which it is supposed to take origin and also in the nervous tissue through which it passes en route to the ventricular cavity as well as in the cerebrospinal fluid itself. Unfortunately it is not easy to separate the colloid product of the pars intermedia from the epithelial cells which surround it or the pars nervosa from the pars intermedia. It is not surprising, therefore, that the results of these inquiries have not yielded wholly concordant results.

Crowe, Cushing, and Homans, as already mentioned, found the colloid obtained from cysts of the pars intermedia, and pure pars intermedia substance scraped off the pars nervosa, equally inactive, and adapted these findings to their hypothesis by assuming that the secretion was activated during its passage through the posterior lobe.

Lewis, Miller, and Matthews (1911) found the posterior part of the anterior lobe, pars intermedia, and pars nervosa, all active as regards the pressor substance, but found the colloid contained in the residual lumen of the gland only active when there was desquamation or autolysis of pars intermedia cells in the colloid. It must be pointed out, however, that the material contained in large cysts is hardly comparable to the colloid observed in small vesicles of the pars intermedia, since the former are lined in large measure by epithelial cells which have vestigial stomodeal characters, and it may be reasonably doubted whether any of the pars intermedia secretion of a specific nature is discharged into these cavities.

Biedl reports that in a number of cases he was able to separate an inactive pars nervosa from the surrounding pars intermedia, though he declares that this can rarely be accomplished on account of the anatomical difficulties involved. Biedl also found pars intermedia substance active in producing a pressor response, thus confirming the claim of Lewis, Miller, and Matthews.

On the basis of these investigations we may now consider that the presence of the pressor substance in the pars intermedia is established but that its relation to the colloid material of the pars intermedia or to the hyaline and granular masses of the pars nervosa remains a subject for further investigation.

The theory of the relation of colloid derived from pars intermedia to the hyaline and granular masses of the pars nervosa rests on the very unconvincing evidence afforded by the similarity in appearance in microscopic preparations, the frequent proximity of hyaline masses to epithelium, the fact that colloid in vesicles increases under experimental conditions which also increase the content of hyaline, and the fact that the vesicles containing colloid have not always a complete epithelial wall.

The theory that hyaline is derived from degenerating epithelial cells which have invaded the pars intermedia does not adequately explain the presence of hyaline in sites where epithelial invasion rarely occurs, as, for example, in the postoptic lamina and in the portions of the pars nervosa remote from the stalk. Furthermore, hyaline is abundant in the hypophysis of animals in which the pars intermedia is sharply delimited from the pars nervosa, and in which there is seldom, if ever, any epithelial invasion.

It is apparent from the foregoing review that Herring's theory of the mode of posterior lobe secretion as expanded by Cushing and his coworkers rests on a very precarious foundation. Its wide acceptance by investigators is doubtless due to the lack of evidence supporting the equally plausible hypothesis that the pars intermedia, like other endocrine organs, secretes its hormones directly into the circulating fluids. It is well to remember, however, that in constructing the Herring hypothesis as modified by subsequent observers certain assumptions are made which are themselves open to doubt. These are as follows: (1) Only epithelial cells are capable of secretory activity. (2) Amorphous masses occupying contiguous situations in histological preparations are chemically similar and are derived from a common source. (3) Masses forming a row in a microscopic preparation do so because they are moving in the same direction towards a common goal. (4) The increase in the amount of a given product under experimental conditions is an exaggeration of a normal process, a dis-

turbance of the equilibrium between secretion and export. In Cushing and Goetsch's silver clip experiment there is, in addition to obstruction of the recessus infundibuli, actual trauma and interference with the blood supply. The really cogent facts in support of the hypothesis are the presence of a specific pressor substance in the cells of the pars intermedia as demonstrated by Lewis, Miller, and Matthews, and confirmed by Biedl, and the presence of pressor substance in the cerebrospinal fluid. The latter is, however, contested by Carlson and Martin, and Wassing, working under Biedl's direction, was unable to demonstrate clearly the presence of vasoconstricting substances in the cerebrospinal fluid by the frog perfusion method.

The present paper is the result of a research undertaken to test the Herring-Cushing hypothesis, as compared with the alternative hypothesis of direct activity of the pars intermedia occupied with direct export of its product into the vascular channels. Before proceeding to a description of the technique employed and of the results obtained, in order that the point of view of the work may be understood, it may be well to point out that glandular cells of different types can only be distinguished from one another with certainty by the observation and study of the actual products of their activity. In the case of a mixed gland such a study must, of necessity, be made by microscopic methods applied to the living gland when possible and supplemented by observations on the precipitability of these products and on their staining reactions, or microchemical reactions if such be available in the fixed condition. The precarious foundation on which identification of gland cells by purely morphologic criteria rests is sufficiently illustrated by the long discussion not yet terminated of the nature of the demilunes of the salivary glands, and the error, most persistent in our text-books of histology and physiology, of describing the pyloric glands as serous glands. Accordingly the first question which presented itself was as follows: Can the pars intermedia cell be distinguished as a physiological unit by the nature of its chemical products from each and every type of cell in the anterior lobe of the hypophysis? This being answered in the affirmative and the characters of this product determined, the next phase of the problem is to determine whether there is any correlation in the embryo between the time of appearance of pressor activity in the hypophysis and that of

the specific product of the pars intermedia cells. Of necessity, the work must include a study of the other parts of the hypophysis as well as of the pars intermedia.

Technique.

Successful handling of hypophysis from the technical standpoint requires that the organ should be removed with the utmost speed as soon as the animal is dead and transferred with as little mechanical injury as possible to a suitable fixing fluid. We have found that postmortem changes proceed with great rapidity, particularly changes which involve the secretory antecedents in the cells of the pars intermedia. Also mechanical injury by pinching with forceps, compression, or traction cause obvious changes in cytological structure. In handling the adult material a certain amount of delay was inevitable on account of the abattoir processes, but usually it was possible to have the hypophysis in the fixing fluid within 5 minutes from the time the hog was hoisted on the wheel. As soon as the animal was bled, the top of the skull and brain were removed and the hypophysis was carefully dissected out of the sella turcica and dropped into a large quantity of fixing fluid. After a lapse of a few minutes, sufficient to stiffen the surface of the organ, the gland was removed with a spoon and carefully divided in the sagittal plane. The halves were allowed to remain in the fixing fluid for 24 hours in the dark, then dehydrated, cleared in bergamot, and imbedded in paraffin according to the usual practice.

For fixing fluids, formaldehyde 40 per cent, aqueous chrome sublimate (Zenker's fluid without acetic acid), and formalin-Zenker (Zenker's fluid without acetic acid nine parts, neutral formalin one part) were employed.

The following staining methods gave the best results.

1. The Acid Fuchsin-Acid Violet Method.—The stain consists of a mixture of 70 cc. of 1.7 per cent solution of Grübler's acid fuchsin in water, to which is added 30 cc. of a saturated aqueous solution of acid violet. After removal of the paraffin by benzene and transfer of the sections through alcohols to water, the sections are stained for 20 to 30 seconds in this mixture. They are then quickly blotted, dehydrated in anhydrous acetone, and cleared in anhydrous benzene. They are

next differentiated in a mixture of three parts of clove oil to one part of absolute alcohol, blotted, washed thoroughly in several changes of benzol, and mounted.

2. The Neutral Safranine-Acid Violet Method.—The dye is prepared as recommended by Bensley but is used in a different way. Sections 4 micra in thickness were cleared in benzene and passed through absolute alcohol to 70 per cent alcohol containing iodine, in which they remained until all mercury deposits were dissolved. The excess iodine was removed by further treatment with 95 per cent alcohol. Then 20 drops of the stock solution of the neutral dye were dropped on the alcohol-covered slide. 4 drops of distilled water were added and the whole was thoroughly mixed on the slide and allowed to remain for 40 to 50 minutes in the open room if the atmosphere was warm and moist or in a moist chamber if it was dry. The excess stain was poured off, the section blotted with a lintless filter paper, passed rapidly through 95 per cent alcohol to remove precipitated dye, dehydrated in absolute alcohol, and cleared in benzene. The sections were differentiated under the microscope with the oil of cloves-absolute alcohol mixture as described above. The beautiful differentiation of the cell elements of the anterior lobe after formalin-Zenker fixation obtained by this method is illustrated in Fig. 1.

3. The Neutral Gentian Method as Described by Bensley.—At this point it may be well to explain that the terms oxyphil, or eosinophil, and basophil as applied to the cells of the anterior lobe are not truly descriptive, since the cells in question stain with either acid or basic stains. It may even be questioned whether the cells described under these names in various animals or the same animal by different authors constitute a strictly comparable group. For this reason, in order to avoid confusion in our own identification of cell types, the following method was adopted. A section was first stained in safranine-acid violet. In this section a group of cells containing representatives of the different types of the anterior lobe was selected, carefully traced with the camera lucida, and each cell identified. The cover-glass was then removed, and the section restained with acid violet and acid fuchsin after extraction of the first stain. The cells were again identified and described, and the process repeated with neutral gentian and other stains.

To determine postmortem change, one half of the gland was placed in the fixing fluid and the other kept for 4 hours in blood serum at 18°C. Sections from the two halves were then compared after the same staining.

Cells of the Anterior Lobe.

In order to establish the fact that the pars intermedia is a functional unit in the hypophysis, it is necessary for comparison to describe and illustrate the cells of the anterior lobe. Five different types of cells are readily distinguished. Three of these are chromophil types and two chromophobe.

1. Chromophil cells.
   (*a*) Type 1. Eosinophil, oxyphil, or acidophil of other investigators.
   (*b*) Type 2. Basophil cells of other investigators.
   (*c*) Type 3. A hitherto undefined cell forming the main mass of the darker band of tissue extending down from the stalk on the anterior surface of the gland.
2. Chromophobe cells.
   (*a*) Chromophobe seen after formalin-Zenker and aqueous chrome sublimate fixation.
   (*b*) Chromophobe seen after formaldehyde fixation.

There is little to be gained by an attempt at description of the cell types since they do not possess cytologic differences either cytoplasmic or nuclear which would suffice to distinguish them with certainty in the absence of specific staining reactions of their organized secretion products. The reader is, therefore, referred to Fig. 1, which shows a section of anterior lobe, formalin-Zenker fixation, stained in safranine-acid violet, showing chromophobes and Types 1 and 2 of chromophil cells. In general, the granules in Type 1 (oxyphil cells) are more diffusely distributed throughout the cell substance than in Type 2 (basophils), and in the latter when the secretory content of the cell is small, it is usually collected into two or more oval or comma-shaped masses which are quite characteristic of this cell in the pig, and serve to identify it whatever the staining method. In both the oxyphils and basophils, so called, alongside of the nucleus may be recognized a mass which never contains secretion granules though in well filled cells it may be obscured by them. This is the sphere. It is always surrounded by a veil of cytoplasm containing many fine mitochondria and in preparations fixed in formalin-Zenker often is detached from the rest of the cytoplasm in part by clear spaces which are apparently similar to the canal-like structure described by Holmgren and others at one pole of the nucleus in epithelial cells. This is the macula described by Addison (1917) in the rat and by Rasmussen (1921) in the woodchuck in the basophil cells. It is not exclusively the property of the basophil cells but may be more conspicuous in these cells in some species. Such a structure is also present in the cells of the pars intermedia. The mitochondria in the oxyphil and basophil cells of the pig tend to be aggregated about the sphere and to some extent about the periphery of the nucleus, though they may

be more dispersed in cells containing a small amount of accumulated secretion, and there are always scattered mitochondria throughout the mass of secretion. These scattered mitochondria mark the location of the residual protoplasm of the cell. The mitochondria are in reality coextensive with the protoplasm and are condensed where the protoplasm is continuous, dispersed where the protoplasm is dispersed by the accumulation in it of secretion product. This may be taken as a criterion of what is essentially cytoplasmic in these cells, as distinguished from the casual and variable products of cell activity. The mitochondrium never intrudes itself into actual spaces of the cytoplasm occupied by a secretion droplet or a group of them. The characters just described, while interesting, are of little use for cell identification, which must depend solely on the properties of the specific secretion products or granules. These differences will be discussed later.

Type 3 of the chromophil cells (Fig. 2) is found in a strap-like layer that extends down the front of the anterior lobe a variable distance from the stalk. It can be clearly recognized in the fresh gland by its darker color and greater transparency than the rest of the anterior lobe. In sections this mass is sharply delimited from the rest of the anterior lobe even though a few anterior lobe elements of the other types may intrude. This division of the anterior lobe contains more connective tissue than the rest, and it is composed of the cells of Type 3, intermingled among which are some other chromophil cells. These cells are especially conspicuous in sections of material fixed in formalin-Zenker and stained with either the neutral safranine compound or acid violet-acid fuchsin. In both cases they stain intensely blue. The cells tend to a polygonal outline in section rather than to the spherical. The nucleus, not markedly different in type from that of the other two types, is placed at one end of the cell, and the cytoplasm presents an irregularly vacuolated appearance. In addition, the cytoplasm is filled with blue-stained granules. These granules are not colored by the stains here used after fixation with formaldehyde, while those of Types 1 and 2 are fairly well preserved.

Among the chromophobe cells in general two types are seen, though it is not certain that they are morphologically distinct. The first includes all of those seen after formalin-Zenker and aqueous chrome

sublimate fixation and is characterized by the fact that the cytoplasm contains no secretion antecedent granules and retains less color than that of the chromophil cells (Fig. 1). The shape is irregular, being determined by external pressures. The second type seen in formalin-fixed preparations is large and tends to be spherical; and the cytoplasm takes a light diffuse stain, but a more intense stain than the smaller chromophobes, and it may contain a few large granules which stain with acid violet. The real nature and relationship of these cells remain undetermined and they are only mentioned here to establish the dissimilarity of the chromophobes and the cells of the pars intermedia.

Cells of the Pars Intermedia.

The cells of the pars intermedia (Fig. 3) (leaving out of consideration the more or less indifferent cells of the residual lumen) are of two sorts, one of which, the secretory cell of the pars intermedia in the strict sense, is different from every other cellular element in the hypophysis and by its presence serves to delimit the pars intermedia. The other type is the colloid-producing cell which is common to the pars intermedia and that part of the gland surrounding the upper portion of the stalk. The colloid-producing cell (Fig. 3, *k*) being of least importance may be disposed of first. It is a small cubical or columnar cell found usually forming the epithelial lining of follicles containing transparent colloid material. It has a basal nucleus and mottled, poorly staining protoplasm, relatively poor in mitochondria. The cells usually show little evidence of secretory activity, but occasionally (Fig. 3) one sees in them small droplets of colloid similar to that in the follicles, showing that the follicular colloid is actually a product of these cells.

The other type of cell, the granular cell of the pars intermedia, constitutes the main bulk of this portion of the gland (Fig. 3). It is of a variable prismatic shape conditioned by mutual pressure, and the nucleus is located near one pole of the cell. Alongside of the nucleus or at some distance from it is a mass of deeply staining cytoplasm of about the same size as the nucleus which we presume contains the sphere and centrioles, though we have not actually demonstrated them. This mass is inconspicuous in preparations in which the secretion has

been stained but very obvious in cells from which the secretion antecedents have been removed, or in preparations in which both the secretion and the mitochondria are stained, for as in other cells of this gland the mitochondrium is coextensive as regards distribution with the real cytoplasm of the cell, and its concentrations in the cell indicate the locations of cytoplasmic condensations as distinguished from those parts of the cell where the cytoplasm is dispersed by the masses of secretion. Around this condensation the mitochondria are abundant in the form of short delicate rods. They are also abundant but less so in the perinuclear cytoplasm and they extend radially from the cytoplasmic mass to the periphery of the cell in lines which coincide with the strands of cytoplasm separating secretion-holding spaces. These same strands of cytoplasm are clearly seen in the cells in question from which the secretion has been dissolved as a result of postmortem change or the solvent action of the fixing fluid. In such cells the cytoplasm presents a coarse meshed network which has recently been well illustrated by Rasmussen (1921) in the cells of similar sort from the pars intermedia of the woodchuck.

The secretion in these cells is a highly labile material which appears in suitably fixed preparations in the form of small granules which are very difficult to stain. The amount in the individual cell is highly variable, and accordingly in preparations the pars intermedia presents a mottled appearance. These granules are not found in the juxtanuclear cytoplasm mass described above. The granules are smaller and stain blue in acid violet but less intensely than those of any of the cells of the anterior lobe. As will be seen later they disappear more rapidly from the cell, as a result of postmortem change, and are less resistant to the solvent action of fixing agents than any of the violet-staining granules of the anterior lobe. In preparations fixed by immersion, frequently only those pars intermedia cells which are near the surface of the piece have their secretion granules preserved, those more centrally placed having undergone in various degrees those changes which generally occur in the pars intermedia of glands kept several hours before fixation. Even here, however, the pars intermedia cell is a distinct secretory type because the granules in the cells of the anterior lobe are little affected.

Postmortem Changes in the Pars Intermedia Cell.

As described under Technique, glands were divided into two equal portions and one was fixed immediately while the other was kept in blood serum for 4 hours, then fixed, and the two lots were compared. In the sections of the piece which had been allowed to undergo this change, no granules were to be found in the cells of the pars intermedia (Fig. 4); instead the cell presented an irregularly vacuolated or rather reticulated appearance due to the withdrawal of the secretion antecedents. This fact possibly explains the variable experimental results obtained by different workers as regards the pressor action of extracts from the pars intermedia and neighboring parts of the hypophysis, for it is probable that so highly soluble a material may diffuse into neighboring sections of the organ to a variable extent, depending on the freshness of the gland and the promptness with which the several parts are separated after removal from the freshly killed animal.

These facts may be summarized as follows: The pars intermedia cells are characterized by their content of secretory granules. These granules are smaller than in any other of the granule-containing cells of the hypophysis. They stain a light shade of blue in the neutral combination of safranine and acid violet, or in the mixture of acid violet and acid fuchsin, or in Mallory's connective tissue stain, after fixation in formalin-Zenker. They are distinguished from all of the chromophil cells of the anterior lobe by the lability of the granules during postmortem change; from both types of blue-staining cells in the anterior lobe by the small size of the granules, and by the fact that the granules are stained lighter and are more diffusely distributed through the protoplasm than the granules of the so called basophils. The pars intermedia cell is distinguished from Type 3 chromophil cell by its lack of vacuoles and its much smaller, lighter stained granules. The cells of the pars intermedia are distinguished from all chromophobes of the anterior lobe by their positive granule content.

The two types of chromophobes are best distinguished from one another by their behavior in sections fixed in strong formaldehyde and formalin-Zenker; in the former large spherical chromophobes are usually abundant.

Study of the Embryonic Hypophysis.

The study of the embryonic hypophysis was undertaken to discover whether there was any relation in time between the appearance of the characteristic pressor effects of the posterior lobe extract and that of the granular secretion antecedents in the cells of the pars intermedia or of hyaline bodies or other structures which have been hypothetically related to the production of the pressor substance.

The technique followed was similar to that employed for the anterior lobe and pars intermedia of the adult.

The cells of the pars intermedia of pig embryos measuring 7.5 cm. in length have large nuclei and very little cytoplasm. The cell boundaries cannot be seen distinctly, and mitoses are abundant. In the 12.5 cm. stage the appearance of the cells is about the same, but vascularization of the mass is indicated by the ingrowth of mesenchyme and blood vessels from the investment of the pars nervosa. The cells are still difficult to delimit. Vascularization is still further advanced in the 17.5 cm. stage, and some of the cells of the deeper layers are increased in size, the increase being due to the expansion of the cytoplasmic body which now begins to show some fine granules (Fig. 5). In some cells these granules are abundant, the cells being fairly packed with them. The granules are minute and have the same staining reactions as the granules of the pars intermedia of the adult. In the later stages a progressively increasing number of cells of the pars intermedia shows this differentiation, and at full term the majority of cells is so differentiated, and the pars intermedia gives the impression of being greater in mass ratio to the other parts of the gland than at any other period of development or growth.

None of the fetal hypophyses contain, so far as we have been able to discover, hyaline bodies.

It is necessary to study serial sections to discover the colloid-containing vesicles, for frequently folds of the pars intermedia, the cavity of which communicates with the residual lumen, present in single sections the appearance of follicles. When actually present the follicles are found to be lined by cylindrical cells with clear non-granular protoplasm and basal nuclei.

These results may now be compared with the physiological action of extracts of the fetal gland as described by Lewis. Lewis found that extracts of the hypophysis of the 7.5 cm. pig were wholly inactive as regards pressor effect even though a very large number of hypophyses was employed in making the extracts. Similarly no definite effects were noted after the injection of extracts of the hypophyses of 12.5 cm. pig fetuses. On the contrary, a marked pressor effect followed injection of extracts made from the hypophyses of 17.5 cm. pigs. The conclusion is obvious—the pressor substance of the posterior lobe of the hypophysis is coincident in time of appearance with the granules or secretory antecedent of the pars intermedia. As an additional reason for assuming a relation between these granules of the pars intermedia cell, it may be pointed out that McCord and Fenger, using the oxytocic method, have found in the new-born calf and lamb that the extracts of the gland were more active per unit weight than in the adult.

CONCLUSIONS.

The bearing of these results on the Herring-Cushing theory of pituitary secretion is apparent. For the first time a true secretion antecedent has been demonstrated in the cells of the pars intermedia, an antecedent which appears in the cells at the same period of development at which active pressor effects may be obtained from the gland extracts. The route of export of this material from the gland to its point of utilization, however, is unknown; it may go by way of the blood or, as required by the Herring theory, by the transneural route to the third ventricle. The objections to the latter conclusion have been amply expanded in the introduction to this paper, but here may be emphasized the fact that the fetal pig hypophysis contains no hyaline bodies. Indeed they are rare in the adult, though there may be seen in the cells of the pars nervosa in the processes of its intrinsic cells, granular deposits which we believe to be the antecedents of the hyaline bodies but which in the pig rarely are discharged and aggregated into discrete masses as in other mammals. The fact that some observers have obtained positive pressor effects from the nervous part of the posterior lobe, exclusive of the pars intermedia, need not weigh very heavily in attempting to trace the course of the secretion,

when we reflect that the difficulties of making such a mechanical separation are almost insuperable, and that the chemical product of the pars intermedia is so soluble and vanishes from the cells so rapidly that it may well be diffusible through the thin membranes which intervene and penetrate post mortem into adjacent parts. We are inclined, therefore, to the view that the secretion leaves the gland by the vascular route rather than by way of the interfibrillar spaces of the pars nervosa.

BIBLIOGRAPHY.

Addison, W. H. F., The cell-changes in the hypophysis of the albino rat after castration, *J. Comp. Neurol.*, 1917, xxviii, 441.

Bensley, R. R., Studies on the pancreas of the guinea pig, *Am. J. Anat.*, 1911–12, xii, 297.

Biedl, A., Innere Sekretion. Ihre physiologischen Grundlagen und ihre Bedeutung für die Pathologie, Berlin and Vienna, 2nd edition, 1913, pt. 2, 143, 144.

Carlson, A. J., and Martin, L. M., Contribution to the physiology of lymph.—XVII. The supposed presence of the secretion of the hypophysis in the cerebrospinal fluid, *Am. J. Physiol.*, 1911–12, xxix, 64.

Crowe, S. J., Cushing, H., and Homans, J., Experimental hypophysectomy, *Bull. Johns Hopkins Hosp.*, 1910, xxi, 127.

Cushing, H., and Goetsch, E., Concerning the secretion of the infundibular lobe of the pituitary body and its presence in the cerebrospinal fluid, *Am. J. Physiol.*, 1910–11, xxvii, 60.

Fenger, F., On the presence of active principles in the thyroid and suprarenal glands before and after birth. (Second paper), *J. Biol. Chem.*, 1912, xii, 55.

Herring, P. T., The histological appearances of the mammalian pituitary body, *Quart. J. Exp. Physiol.*, 1908, i, 121; The effects of thyroidectomy upon the mammalian pituitary. Preliminary note, 281.

Holmgren, E., VI. Neue Beiträge zur Morphologie der Zelle, *Ergebn. Anat. u. Entwcklngsgesch.*, 1901, xi, 274.

Howell, W. H., The physiological effects of extracts of the hypophysis cerebri and infundibular body, *J. Exp. Med.*, 1898, iii, 245.

Lewis, D., The appearance of the pressor substance in the fetal hypophysis, *J. Exp. Med.*, 1916, xxiii, 677–678.

Lewis, D., Miller, J. L., and Matthews, S. A., The effects on blood-pressure of intravenous injections of extracts of the various anatomical components of the hypophysis, *Arch. Int. Med.*, 1911, vii, 785.

McCord, C. P., The occurrence of pituitrin and epinephrin in fetal pituitary and suprarenal glands, *J. Biol. Chem.*, 1915, xxiii, 435.

Oliver, G., and Schäfer, E. A., On the physiological actions of extracts of pituitary body and certain other glandular organs, *J. Physiol.*, 1895, xviii, 277.

Rasmussen, A. T., The hypophysis cerebri of the woodchuck (*Marmota monax*) with special reference to hibernation and inanition, *Endocrinol.*, 1921, v, 33.

EXPLANATION OF PLATE 5.

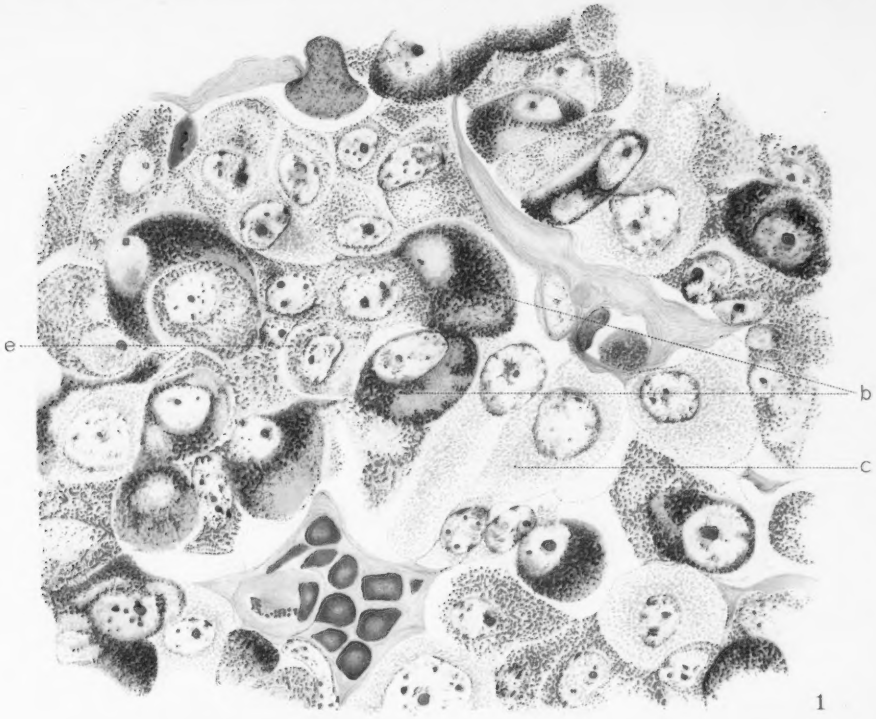
FIG. 1. Anterior lobe of pig hypophysis fixed in formalin-Zenker and stained with safranine-acid violet. (*e*) chromophil cell, Type 1 (eosinophil of authors); (*b*) chromophil cells, Type 2 (basophil of authors); (*c*) chromophobe cell.

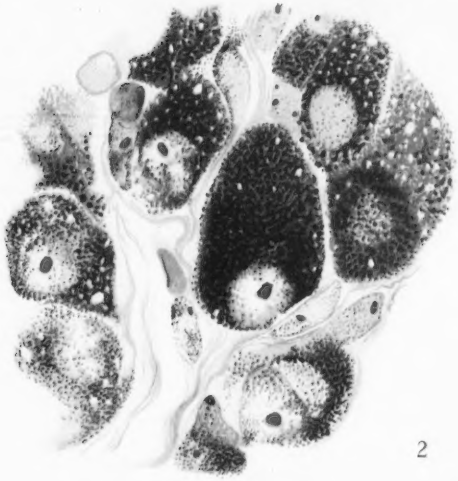
FIG. 2. Chromophil cells, Type 3, from the anterior border of the hypophysis of the pig stained with safranine-acid violet.

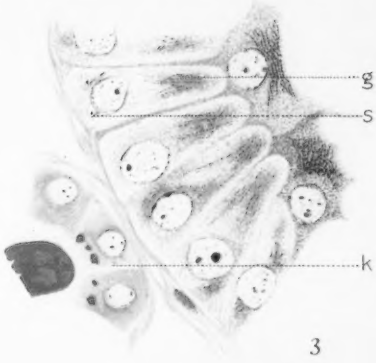
FIG. 3. Pars intermedia of adult pig hypophysis. Formalin-Zenker; aniline-acid fuchsin and phosphomolybdic water blue. (*k*) part of the wall of a follicle containing colloid, showing colloid cells with colloid droplets at their free borders; (*g*) granular cell of the pars intermedia showing the blue-stained secretion and cytoplasmic mass containing mitochondria; (*s*) granular secretion.

FIG. 4. Two of the chromophobes of the anterior lobe as seen after formalin fixation; acid fuchsin-acid violet stain.

FIG. 5. Group of cells from the pars intermedia of the hypophysis of a fetal pig of 17.5 cm. Four of the cells already have the differentiated characters of the adult pars intermedia. Others are undifferentiated. Formalin-Zenker; acid fuchsin-phosphomolybdic water blue. (*g*) granular secretion antecedent.







A. HOEN & CO. BALTIMORE.

(Maurer and Lewis: Pars intermedia of hypophysis.)